# COMPREHENSIVE STUDY OF THE OXIDATIVE STABILITY OF THE MOST CONSUMED ULTRA-PROCESSED FOODS IN THE USA

By

Lisaura Maldonado-Pereira

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Chemical Engineering – Doctor of Philosophy
Biosystems Engineering – Dual Major

2021

## ABSTRACT

COMPREHENSIVE STUDY OF THE OXIDATIVE STABILITY OF THE MOST CONSUMED ULTRA-PROCESSED FOODS IN THE USA

By

Lisaura Maldonado-Pereira

Ultra-processed foods (UPFs) are characterized for being inexpensive, highly processed, rich in calories, but low in some essential micronutrients such as minerals and vitamins. Nearly 60% of the calories consumed by the average American come from UPFs (Martínez Steele et al., 2016). Processing of food plays a crucial role in the overall chemical safety of the American diet (also known as the Western diet) since different manufacturing conditions promote the formation of unintended and harmful compounds in the final product. This is the case of cholesterol oxidation products (COPs) which have been associated with the development of several chronic diseases. COPs are derived from the oxidation of cholesterol and can be triggered by different parameters such as light, heat, radiation, metal ions, and other agents. Until this study, there has been no database of these compounds in the Western diet and their level of exposure of the US population.

A total of 63 UPFs were tested. Fatty acids, cholesterols and its oxidation products, tocopherols and phytosterols were comprehensively assessed by chromatographic means. Oxidative status of the Western diet was evaluated by the quantification of secondary oxidation products such as malondialdehyde (MDA). Twelve main COPs (7α-OH, 7β-OH, 4β-OH, 5,6α-Epoxy, 5,6β- Epoxy, 7-Keto, Triol, 6-Keto, 20α-OH, 22-OH, 24-OH, and 25-OH), and other sterols (cholesterol, phytosterols, and tocopherols) were detected. An assessment of the level of exposure of COPs was performed using the Stochastic Human Exposure and

Dose Simulation (SHEDS) developed by the US Environmental Protection Agency (EPA) (EPA, 2020).

Forty-four percent of the samples showed a different fat content than those reported on the food item's nutritional label. Moreover, 78% of the UPFs showed a higher cholesterol content than the reference value from their nutritional label. Twenty-six percent of fast food (FF) meals showed a high PUFA content which is a type of healthy fat that improves cardiovascular health (Harris, 2007; Lu et al., 2011), brain function, and overall health during pregnancy (Koletzko et al., 2008). Saturated fatty acid (SFA) content in UPFs was directly related to the food item's price. β-Sitosterol was the most abundant phytosterol. However, differences in concentrations were observed depending on the food matrix and ingredients added to the food item throughout its preparation. Similarly, total COP content varied among food matrices and ingredients added. This means that food matrix, ingredients and cooking conditions employed in the product's confection play an important role in the distribution of these sterols. Lastly, infants (6-12 months) could be exposed to upwards of 309.56 mg/kg/6 mo. (0.0031 mg/kg/6 mo.). Since there is no study addressing the effects of COPs on infants' health, no assumption could be done to determine if it should be considered a health risk.

This study provides a complete overview of the oxidative lipid status of the most popular UPFs in the Western diet as well as an assessment of the exposure level of these compounds in one of the most vulnerable groups: infants. Nutritional quality and dietary patterns seem to be jeopardized by prices and popularity of UPF meals, resulting in a public health issue that should be addressed.

This dissertation is dedicated to my family and Obie.
Thank you for always believing in me.

# ACKNOWLEDGEMENT

Life has mysterious ways to teach each of us the meaning of hard work, resilience, group support, perseverance, compassion, and courage. Many people have been key to the success of this personal and yet collective milestone.

I would like to express my infinite gratitude to several people, starting with my mentor, advisor, and second mother, Dr. Ilce Medina-Meza. Thank you for believing in me. Your active guidance throughout the completion of my doctoral degree has been my compass in this winding adventure. You are an exceptional role model and have helped to shape me into a better professional and academic.

Also, I will always be grateful to my graduate committee for their willingness to help me throughout this journey. Lastly, this triumph could not be possible without the support of Dr. Percy Pierre. Thank you for allowing me to stay in school and continue my doctorate degree. Your encouragement and help during difficult times are the base of this achievement.

I am eternally thankful to both of my Departments (the Chemical Engineering Department and the Biosystem and Agricultural Department) for supporting me throughout my entire doctoral program and always putting the student's interest first. Finally, I extend my appreciation to those who could not be mentioned here but have played their role to inspire, help, and continuously encourage me to give my all in this process.

Lisaura Maldonado-Pereira

TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# KEY TO ABBREVIATIONS

| | |
|---|---|
| COPs | cholesterol oxidation products |
| CHOL | cholesterol |
| ROS | reactive oxygen species |
| 7-OH | 7-hydroxycholesterol |
| 7-KETO | 7-ketocholesterol |
| 5,6α-EPOXY | 5α,6α-epoxycholesterol |
| 5,6β-EPOXY | 5β,6β- epoxycholesterol |
| LDL | low density lipoprotein |
| HDL | high density lipoprotein |
| VLDL | very low-density lipoprotein |
| LXR | liver X receptor |
| 7α-OH | 7α-hydroxycholesterol |
| 7β-OH | 7β-hydroxycholesterol |
| 24S-OH | 24S-hydroxycholesterol |
| 27-OH | 27-hydroxycholesterol |
| AR | androgen receptor |
| MS | multiple sclerosis |
| 25-OH | 25-hydroxycholesterol |
| 20-OH | 20-hydroxycholesterol |
| TRIOL | Cholesteane-3β, 5α, 6β-triol |
| HD | Huntington's disease |
| AD | Alzheimer's disease |
| MCI | mild cognitive impairment |
| SFA | saturated fatty acids |
| MUFA | monosaturated fatty acids |
| PUFA | polyunsaturated fatty acids |

| | |
|---|---|
| DHA | docosahexaenoic acid |
| ARA | arachidonic acid |
| FF | fast food |
| RTE | ready-to-eat |
| UPFs | ultra-processed foods |
| PFs | processed foods |
| TDS | total dietary study |
| FDA | Food and Drug Administration |
| ACAT | acyl-coenzyme A cholesterol acetyltransferase |
| UHT | ultra-high temperature |
| U937 | Human lymphocyte cells |
| MCF7 | Human mammary gland, breast; derived from metastatic site: pleural effusion |
| CYP | Cytochrome P450 enzyme family |
| HUVEC | Human umbilical vein endothelial cell |
| POPs | Phytosterol Oxidation Products |
| IF | Infant Formula |
| SHEDS | Stochastic Human Exposure and Dose Simulation |
| SHEDS-HT | Stochastic Human Exposure and Dose Simulation – High Throughput |

# CHAPTER 1 : IMPORTANCE OF COPS IN FOOD TOXICITY

The content of this chapter has been previously published in the Food and Chemical Toxicology Journal. DOI: 10.1016/j.fct.2018.05.059

## 1.1 INTRODUCTION

Cholesterol is a key component of mammalian cells with a crucial role in different biological processes. It is a precursor of a variety of hormones, vitamin D and bile acid production cycle (Vesa M Olkkonen, Gylling, & Ikonen, 2017). Cholesterol is synthetized endogenously in the body in amounts necessary to sustain life. The presence of reactive oxygen species (ROS) due to cell oxidative stress or exposure to radical species increases cholesterol susceptibility to oxidation (I. G. Medina-Meza & C. Barnaba, 2013; L. L. Smith, 1996). This non-enzymatic oxidation occurs at the C5-C6 double bond via nucleophilic attack by radicals and other reactive species. Cholesterol's sidechain is also susceptible of radical attack (Iuliano, 2011; L. L. Smith, 1996). ROS-mediated oxidation can be triggered by light (Maerker & Jones, 1993a; Ilce Gabriela Medina-Meza & Barbosa-Cánovas, 2015; Ilce Gabriela Medina-Meza, Rodriguez-Estrada, Sergio Garcia, & Lercker, 2012; Medina-Meza, Rodriguez-Estrada, Lercker, Barnaba, & García, 2014), Heat (Chien, Wang, & Chen, 1998; D. Derewiaka & Molinska nee Sosinska, 2015), Radiation (Maerker & Jones, 1993a), metal ions (Hur, Park, & Joo, 2007b; Jo, Ahn, & Lee, 1999) and other agents that have the ability to lower the energetic requirements for the reaction to occur (Beltran, Pla, Capellas, Yuste, & Mor-Mur, 2004; I. Medina Meza & Barnaba, 2013). Cholesterol oxidation produces a group of molecules that preserve the steroidal motif of the parent compound, but with an

additional hydroxyl, ketone, or epoxy group (Savage, Dutta, & Rodriguez-Estrada, 2002) (Figure 1-1).

They are commonly known as cholesterol oxidation products (COPs), and depending on the site of oxidation, COPs can be distinguished as α-ring, β-ring, or side-chain oxidation products (Figure 1-1 and Table 1-1). Each of these molecules have demonstrated biochemical, biological, and even biophysical activities in mammalian cells and subcellular components (Gabriella Leonarduzzi et al., 2005). Endogenous COPs have been considered as key intermediates in bile acid and steroid hormone biosynthesis or as autoxidation

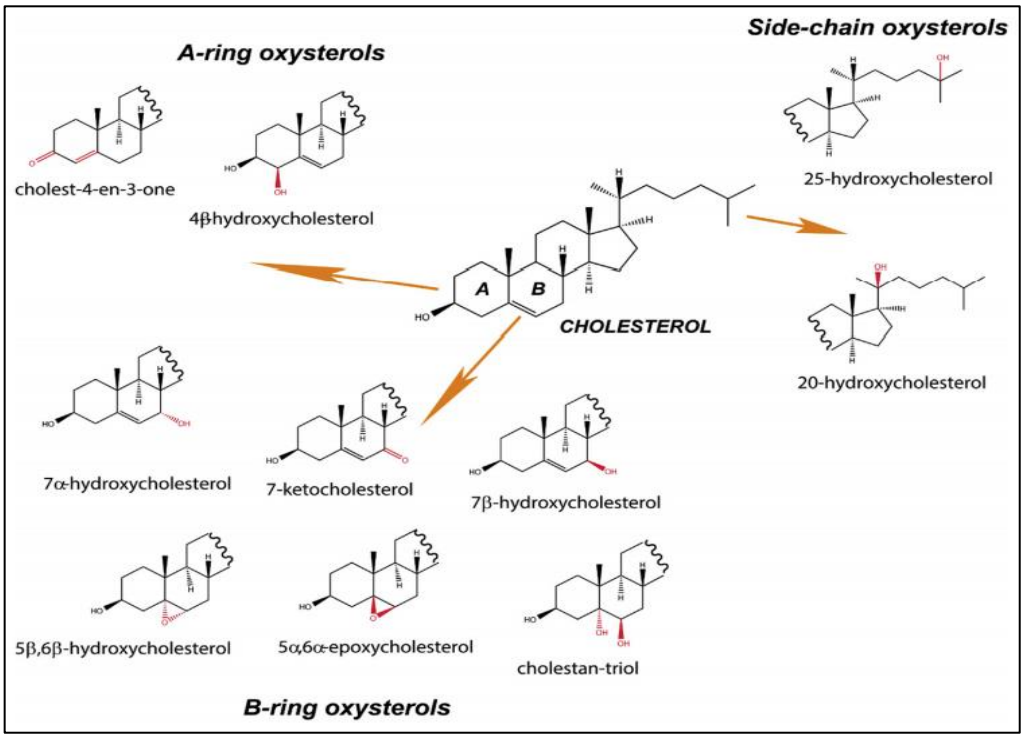


***Figure 1-1:*** *Diagram obtained from Maldonado-Pereira et al. 2018 which explains the different structures of the main COPs according to their site of oxidation*

products in tissues, especially under conditions of oxidative stress (Ikegami et al., 2014; Vesa M. Olkkonen, Béaslas, & Nissilä, 2012; Vaughn et al., 1997). On the other hand, exogenous COPs are mainly derived from diet by consumption of high cholesterol-containing foods. This intake results in the accumulation of COPs in several organs and tissues, as it

has been extensively reported in previous studies (Biasi et al., 2013; Miyoshi, Iuliano, Tomono, & Ohshima, 2014; Ogino et al., 2007; Ros, 2000). Animal food products including meat and meat products (K. Al-Ismail, 2002; KM Al-Ismail, Herzallah, & Humied, 2007; Bilska, Rudzińska, Kowalski, & Krysztofiak, 2009; Britt, 1998; Y. Chen, T., Stephen, & Bing Huei, 2012; Grau, Codony, Grimpa, Baucells, & Guardiola, 2001; Novelli et al., 1998; Osada, Kyoichi, Shinichi, Shingo, & Michihiro, 2000; Zubillaga M. P. and Maerker, 1991); (Hur, Lee, & Lee, 2015), fish (Echarte, Conchillo, Ansorena, & Astiasarán, 2004; Hernández, 2014; Osada et al., 1993; Paola Zunin, Boggia, & Evangelisti, 2001), eggs and egg products (L. J. Chen, Lu, Chien, & Chen, 2010), cheese (Nourooz-Zadeh, 1988), milk (Calderón-Santiago, Peralbo-Molina, Priego-Capote, & Luque de Castro María, 2012; Dionisi, Golay, Aeschlimann, & Fay, 1998) and other dairy products (Angulo, Romera, Ramirez, & Gil, 1997; Gorassini, Verardo, Fregolent, & Bortolomeazzi, 2017; Kumar, 1992; Mariutti & Bragagnolo, 2017; Nielsen, 1995; X. D. Sun & Holley, 2010) are the major source of dietary COPs (A Grandgirard, Guardiola, Dutta, Codony, & Savage, 2002; Hur, Park, & Joo, 2007a; Ogino et al., 2007). The content of COPs in food has been largely overlooked, despite a consistent body of evidence on their biological and pathological activities in humans. COPs are unavoidable and unintentionally formed during food processing, storage, handling and even household preparations, making human exposure a tangible risk.

This chapter will discuss the gap between the scientific evidence of COPs formation in biological systems, and the chemical exposure and toxicological significance through dietary intake. First, data regarding the occurrence of COPs in foods and other biological matrices will be reviewed. Second, the compound-specific biological and pathological significance will be addressed.

***Table 1-1*:** Classification table of the origin (endogenous and exogenous) of the most detected COPs published in Maldonado-Pereira et al., 2018.

| Common name | Abbreviation | Location* | | | Formation Pathway | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | *Endogenous origin* | | | *Exogenous origin* |
| | | Side-chain | Ring A | Ring B | Enzymatic formation/ Enzyme** | | Non-enzymatic formation (ROS) | Autoxidation /Dietary Intake |
| 27-hydroxycholesterol | 27-OH | X | | | X | 27-hydrolase (CYP27A1) | | |
| 24S-hydroxycholesterol | 24S-OH | X | | | X | 24-hydrolase (CYP46A1) | | |
| 22-hydroxycholesterol | 22-OH | X | | | X | 22-hydrolase (CYP11A1) | | |
| 25-hydroxycholesterol | 25-OH | X | | | X | 25-hydrolase (Ch25h), CYP3A4 | X | X |
| 20α-hydroxycholesterol | 20-OH | X | | | | | | X |
| 4β-hydroxycholesterol | 4β-OH | | X | | X | CYP3A4 | | X |
| 7α-hydroxycholesterol | 7α-OH | | | X | X | 7α-hydrolase (CYP7A1) | X | X |
| 7β-hydroxycholesterol | 7β-OH | | | X | | | X | X |
| 7-ketocholesterol | 7-keto | | | X | | | X | X |
| Cholesterol-5α,6α-epoxide | 5,6α-epoxy | | | X | | | X | X |
| Cholesterol-5β,6β-epoxide | 5,6β-epoxy | | | X | | | X | X |
| Cholesterol-3β, 5α, 6β-triol | Triol | | | X | | | X | X |
| 5,6-secosterol | secosterol | | | X | | | | X |
| 3β,5α-dihydroxycholest-7-en-6-one | DHCEO | | | X | X | 7-dehydrocholesterol reductase (DHCR7) | | |

*(Tai et al., 1999) (Mast et al., 2011; Xu et al., 2012).
**(Aldini et al., 2010) (Tai et al., 1999).

Subsequently, the factors that enhance their formation in food products will be analyzed. Finally, the importance of an accurate estimation of these compounds' fate through a risk exposure assessment will be highlighted. Overall, this chapter will strengthen the need of a holistic approach on cholesterol oxidation and COPs, an emerging field already known as "cholesterolomics", which will be helpful in the assessment of novel chemical biomarkers for food safety and toxicology.

## 1.2 OCCURRENCE OF COPS IN BIOLOGICAL SYSTEMS

### 1.2.1 Absorption and transport of COPs

The fate of dietary cholesterol and cholesterol-derivatives has been studied in different animal models, including humans. Dietary cholesterol appears in the lumen of the intestine associated with triglycerides and phospholipids in lipid emulsion. These lipid

micelles are digested by lipases, and cholesterol is released for its transport to the brush border of the small intestine for absorption by the mucosal cells (Shoshana Rozner and Nissim, 2006). Cholesterol is absorbed in the human small intestine through ABCG5/ABCG8 and NPC1L1 transporters, which effluxes a portion of the absorbed cholesterol back into the small intestinal lumen and drives the cholesterol uptake in the enterocytes (Shoshana Rozner and Nissim, 2006). On the contrary, the absorption pathway of COPs has not been characterized in its completeness due to their chemical diversity, polarity, and abundance in the diet. Studies have shown that COPs are absorbed in the small intestine at a lower rate than cholesterol (−66%) (K. Osada, Sasaki, & Sugano, 1994), probably due to their lower solubility in micelles. Cholesterol, fatty acids and eventually COPs are then assembled into large intestinal lipoproteins, called chylomicrons. Cholesterol is esterified by the acyl-coenzyme A cholesterol acetyltransferase (ACAT) before incorporation into the chylomicrons. Intragastric administration of a mixture of COPs in rats revealed that their absorption is related with changes in the composition of lymph chylomicron after 2–4h (Emanuel, Hassel, Addis, Bergmann, & Zavoral, 1991). The authors also found a time-correlation between the incorporation of individual COPs' species into the lymph chylomicrons. 7-OH isomers were early incorporated (3 h), followed by 7-keto and 5,6α-epoxy at 4 and 5 h, respectively (Vine et al., 1997, 1998). 7-OH isomers and 7-keto are the COPs commonly identified in lipoproteins (Kuver, 2012). The highest levels of COPs are present in low-density lipoproteins (LDLs) and to a lesser extent in high-density lipoproteins (HDLs) and very-low-density lipoproteins (VLDLs) (Vaya et al., 2001; A. Vejux & G. Lizard, 2009). Huang et al. (2015) suggested the revision of the cholesterol transport hypothesis since several epidemiological studies demonstrated the cholesterol efflux (reverse cholesterol transport) plays an important role in the absorption and removal of excess of cholesterol in plasma.

Notwithstanding, no information is still provided about the implication of COPs in the absorption of cholesterol.

### 1.2.2 Biological and pathological activities of COPs

In the last decades, there has been mounting evidence of COPs exerting biological and pathological activities in both in vitro and in vivo systems, with potential health concerns for humans (Gabriella Leonarduzzi et al., 2005). Several studies have demonstrated that COPs can exert pro-inflammatory (Biasi et al., 2013; Lemaire-Ewing et al., 2005; Miyoshi et al., 2014; Virginio et al., 2015), pro-oxidant (Biasi et al., 2009; Mariutti & Bragagnolo, 2017; Seet et al., 2009), pro-fibrogenic (Gargiulo, Gamba, Testa, Leonarduzzi, & Poli, 2016) and pro-apoptotic (Colles, Maxson, Carlson, & Chisolm, 2001; A. Vejux & G. Lizard, 2009) activities in several cell lines (Raza et al., 2016; A. Vejux & G. Lizard, 2009; Zarrouk et al., 2014). COPs have also shown specific deleterious properties such as cytotoxicity, mutagenicity (Sevanian & Peterson, 1986), carcinogenicity (Homma et al., 2004), Alzheimer's disease (Marwarha & Ghribi, 2014), Parkinson's disease (Bjorkhem et al., 2013; C. Y. Lee, Seet, Huang, Long, & Halliwell, 2009; Leoni & Caccia, 2011), age-onset macular degeneration (Javitt & Javitt, 2009) as well as cataracts (Girão, Mota, Ramalho, & Pereira, 1998), osteoporosis (H. Liu, Yuan, Xu, Zhang, & Wang, 2004), colon carcinoma (Biasi et al., 2009; Roussi et al., 2005), prostate cancer (Fukuchi et al., 2004; Homma et al., 2004; Kulig, Cwiklik, Jurkiewicz, Rog, & Vattulainen, 2016), and breast cancer (Cruz et al., 2010; Gruenke et al., 1987; Lappano et al., 2011; Nelson, Chang, & McDonnell, 2014; Wu et al., 2013). The potential relationship between COPs and several chronic diseases is summarized in Table 1-2, accompanied by a brief description of the speculated mechanism(s). For more information about other chronic diseases and details regarding

each specific COPs, go to APPENDIX A - Table 2. Due to the demonstrated effects of COPs in diffused and emerging human pathologies, there is an imperative demand on more accurate and systematic information about COPs' intake in targeted populations (i.e., infants, elder people, etc.).

***Table 1-2:*** *Summary of the table reported in Maldonado-Pereira et al., 2018 which describes the relationship between COPs and different chronic diseases. Dietary COPs are highlighted.*

| CARDIOVASCULAR DISEASES | | | |
|---|---|---|---|
| ATHEROSCLEROSIS | | | |
| **Oxysterol** | **Affected tissue** | **Effect** | **Reference** |
| 27-OH | Peripheral artery | - Oxidative stress (mixture)<br>- Inflammation (alone)<br>- Endothelial dysfunction/cell phenotype changes<br>- Acts as agonists of liver X receptors (LXRs) | Virginio et al., 2015 |
| | Carotid | | Leonarduzzi et al., 2007 |
| | Aorta | | Upston et al., 2002 |
| | Carotid/Aorta | | Garcia-Cruset et al., 2001 |
| | Coronary | | Vaya et el., 2001 |
| 24S-OH | ND | - Apoptosis (alone)<br>- Acts as agonists of liver X receptors (LXRs) | Alkazemi et al., 2008, Gargiulo et al., 2015 |
| 22-OH | ND | - Vascular calcification (alone/mixture)<br>- Acts as agonists of liver X receptors (LXRs) | Alkazemi et al., 2008, Gargiulo et al., 2015 |
| 25-OH | Peripheral artery | - Oxidative stress<br>- Inflammation (alone/mixture)<br>- Apoptosis (alone)<br>- Endothelial dysfunction/cell phenotype changes<br>- Vascular calcification<br>- Acts as agonists of liver X receptors (LXRs) | Alkazemi et al., 2008, Virginio et al., 2015 |
| 7β-OH | Carotid | - Oxidative stress<br>- Inflammation (alone)<br>- Apoptosis (alone)<br>- Endothelial dysfunction/cell phenotype changes (alone) | Helmschrodt et al., 2013, Leonarduzzi et al., 2007 Micheletta et al., 2004, Iuliano et al., 2003, Carpenter et al., 2003, Vaya et al., 2001 |
| | Carotid/Aorta | | Garcia-Cruset et al., 2001 |
| | Coronary | | Vaya et al., 2001 |
| 7α-OH | Carotid | - Oxidative stress<br>- Inflammation<br>- Apoptosis<br>- Endothelial dysfunction/cell phenotype changes | Helmschrodt et al., 2013 |
| | Carotid/Aorta | | Garcia-Cruset et al., 2001, Alkazemi et al., 2008 |
| | Coronary | | Vaya et al., 2001 |

*Table 1-2 (cont'd)*

| CARDIOVASCULAR DISEASES | | | |
|---|---|---|---|
| **ATHEROSCLEROSIS** | | | |
| 7-Keto | Carotid | - Oxidative stress<br>- Inflammation<br>- Apoptosis<br>- Endothelial dysfunction/cell phenotype changes<br>- Acts as agonists of liver X receptors (LXRs) | Helmschrodt et al., 2013, Leonarduzzi et al., 2007, Micheletta et al., 2004, Iuliano et al., 2003, Vaya et al., 2001, Upston et al., 2002 |
| | Carotid/Aorta | | Garcia-Cruset et al., 2001 |
| | Coronary | | Vaya et al., 2001 |
| 5,6α-Epoxy | Carotid | - Oxidative stress<br>- Inflammation (alone)<br>- Apoptosis (alone)<br>- Endothelial dysfunction/cell phenotype changes (alone) | Helmschrodt et al., 2013, Vaya et al., 2001 |
| | Carotid/Aorta | | Garcia-Cruset et al., 2001 |
| | Coronary | | Vaya et al., 2001 |
| 5,6β-Epoxy | Carotid | - Oxidative stress (alone/mixture)<br>- Inflammation (alone/mixture)<br>- Apoptosis (alone/mixture) | Helmschrodt et al., 2013 |
| | Carotid/Aorta | | Vaya et al., 2001 |
| | Coronary | | Garcia-Cruset et al., 2001, Vaya et al., 2001 |
| Triol | Carotid | - Oxidative stress<br>- Inflammation<br>- Apoptosis<br>- Endothelial dysfunction/cell phenotype changes (alone)<br>- Vascular calcification | Helmschrodt et al., 2013 |
| **NEUROLOGICAL DISEASES** | | | |
| **MULTIPLE SCLEROSIS** | | | |
| **Oxysterol** | **Affected tissue** | **Effect** | **Reference** |
| 25-OH | Central nervous system (neurons) | Damage to the myelin in the central nervous system | Mukhopadhyay et al., 2017 |
| 7α-OH | | | |
| 7-Keto | | | |
| **CANCER** | | | |
| **BREAST CANCER** | | | |
| **Oxysterol** | **Affected tissue** | **Effect** | **Reference** |
| 27-OH | Breast | Promotes the proliferation of the estrogen receptor (ER) positive breast cancer cell lines in vitro. | Cruz et al., 2010 |
| | | | Wu et al., 2013; Nelson et al., 2014 |
| 25-OH | | - Induce the recruitment of Era to the ERE site located in the pS2 promoter sequence in MCF7 cells.<br>- Induce proliferative effects in a dose dependent manner. | Lappano et al., 2011 |

Forty five percent of Americans have suffered from some type of chronic illness (2014), and according to the Agency for Healthcare Research and Quality (AHRQ), chronic

diseases (including those COPs related diseases), are the most prevalent healthcare issues in the United States (NIH, 2017; Quality, 2020). These conditions include arthritis, asthma, cancer, cardiovascular (heart) disease, depression, and diabetes, though these are only a few of many chronic illnesses that negatively affect the lives of Americans (Quality, 2020). The World Health Organization's Department of Evidence and Research shows that many deaths have been caused by chronic diseases some of them closely related with COPs.

From a physiological point of view, individual COPs play an important role in the development of several age-related diseases because of a decrease in oxidative defenses and control of the level of oxysterols, as it is observed in hypercholesterolemia (Micheletta et al., 2004; Zarrouk et al., 2014). Hypercholesterolemia not only can result in coronary heart disease, stroke, and peripheral arterial disease, type 2 diabetes, and hypertension atherosclerosis but high amounts of cholesterol in the body increase the amounts of COPs in the body as well (Carpenter et al., 2003; Garcia-Cruset, Carpenter, Guardiola, Stein, & Mitchinson, 2001; Gargiulo et al., 2015; Menéndez-Carreño et al., 2011; Murakami et al., 2001; NIH, 2017; Shim et al., 2008; Upston et al., 2002; Vaya et al., 2001). Given the different biochemical and biophysical properties of COPs in altering specific cell compartments and enzyme/receptors, several cells and tissue-specific pathological effects have been performed. Different studies have shown that some COPs adversely affect the function of some major organs like brain, eyes, heart, and vessels (Bretillon et al., 2007; Gargiulo et al., 2016). Understanding how the structure and chemistry of COPs define their specific function and mechanism(s) of action inside the human body could help to identify how COPs affect targeted tissues and the exact conditions that triggers their formation. Many hypotheses have arisen to explain the specific role that COPs play in the pathology of a variety of diseases (Table 1-2).

### 1.2.3 COPs as biomarkers of chronic diseases

Experimental evidence points out that COPs can adversely affect the function of some major organs, but the interaction and mechanisms of COPs in the specific affected organ is still not well understood. (C. Y. Lee et al., 2009) have suggested that the apparent association of specific COPs with people more prone to suffer of these chronic diseases could be beneficial, as COPs could be used as biomarkers for these diseases (Aldini, Yeum, Niki, & Russell, 2010; Alkazemi, Egeland, Vaya, Meltzer, & Kubow, 2008; Iuliano et al., 2003). As an example, Linseisen's work (Linseisen, Wolfram, & Miller, 2002) associated the presence of 7β-OH with lung cancer risk revealing high risk estimates, which was lately confirmed by Kang and co-workers (Kang et al., 2005). Abnormal cholesterol biosynthesis has been associated to tissue and fluid accumulation of 7β-OH in Smith-Lemli-Optiz syndrome (L. Xu et al., 2011; Libin Xu et al., 2012). (Yoshida et al., 2003) found some sort of connection between oxysterols and the hepatic bile disease, proposing that the most common oxysterols in gallstones may be generated in the gallbladder in response to bacterial infection. Leoni and Caccia (Leoni & Caccia, 2011) mentioned the use of 24S-OH as a possible surrogate biomarker by the number of metabolically active neurons located in the grey matter of the brain due to its reduced levels of 24-hydroxylase (CYP46A1) with subsequent reduction in the formation of 24S-OH and lower efflux from the brain to the circulation (Leoni et al., 2013; Leoni et al., 2008). These levels of 24S-OH in the circulation were found to be significantly reduced compared to controls in different neurodegenerative diseases such as Alzheimer's disease (AD), Multiple sclerosis (MS) and Huntington's disease (HD) (Leoni & Caccia, 2011; Lütjohann et al., 2000). They also associated higher levels of 27-OH with the processing and deposition of Aβ peptides in both AD and Mild Cognitive Impairment (MCI) patients. In individuals suffering multiple sclerosis (MS), elevated

concentrations of 7-keto have been detected in the cerebrospinal fluid; however, mechanistic evidence has been reported only for the induction of neuronal damage via the activation and migration of microglial cells (Anne Vejux & Gérard Lizard, 2009). Other COPs derived from cholesterol auto-oxidation (7α-OH, 7β-OH, and 25-OH) also present in the central nervous system are known to cause damage to the myelin; however, a complete understanding of the associated molecular mechanisms is still missing (Mukhopadhyay et al., 2017). Huntington's disease (HD), a disease that causes neuronal dysfunction and death, has been associated to transcriptional repression, oxidative injury and mitochondrial dysfunction provoked by accumulation of enzymatic 24S-OH and 27-OH in brain.

Analyses performed postmortem on brain tissue of HD patients showed a 60% decrease in 24S-OH, 30% increase in cholesterol, and 50–70% increase in 7-keto and 7β-OH (both derived predominantly from ROS action on cellular cholesterol), suggesting a significant inhibition of cholesterol metabolism and the contribution of oxidative stress in HD pathology (Kreilaus, Spiro, McLean, Garner, & Jenner, 2016). Lastly, for Parkinson's disease, (Bosco et al., 2006) stated that enzymatic oxysterols (mainly 24S-OH and 27- OH, and secosterol) are particularly responsible for causing α-synuclein aggregation and destruction of dopamine-containing neurons. All these relationships could help to use COPs as biomarkers in different human diseases. Nevertheless, the concurrence of biochemical action (i.e., cytochrome P450s metabolism) and oxidative stress (i.e., cholesterol autooxidation) in the formation of individual COPs make challenging a severe and robust identification of disease-specific markers. Thus, although a potential use of COPs as biomarker exists, this cannot prescind from an overall assessment of individual physiology and oxidative conditions.

### 1.2.4 COPs as a Promoter of Cellular Apoptosis

Even though there is a knowledge gained over the years about the mechanisms by which COPs exert their pathologic effects and considering that cholesterol oxidation generates more than 70 derivatives, volatile organics and $H_2O_2$ (L. L. Smith, 1996), we still lack an exhaustive comprehension of the mechanisms of formation and action. It is worth to mention that there is a significant overlap between COPs generated via enzymatic pathways (mainly P450's associated metabolism of cholesterol and other COPs' precursors) and COPs derived from auto-oxidation of cholesterol (Table 1-1).

There is a copious amount of literature demonstrating COPs' cytotoxicity on several cell lines and in vivo. The cytotoxic activity of COPs is mainly derived from their ability to induce apoptosis through several mechanisms. An exhaustive discussion of biological action of COPs is beyond the scope of this chapter, and the author suggests a few compelling reviews already published. Here, we will summarize the significant findings regarding the role played by those COPs which are derived from diet.

Apoptosis is a programmed cell death, a critical biological process involved in ontogenesis and tissue homeostasis. Dysregulation of apoptosis can promote important diseases, including cancer and atherosclerosis (Lopez, 2015; Tracie Seimon and Ira, 2009). In general terms, cell apoptosis occurs via two pathways: the mitochondrial or intrinsic pathway and the death receptor-dependent or extrinsic pathway (G. Leonarduzzi, Poli, Sottero, & Biasi, 2007; Sinéad Lordan and John, 2009). The first leads to direct activation of caspases, a family of cytosolic proteases that transmit the apoptotic pathway by making specific protein cleavages (Wolf & Green, 1999). On the other hand, the mitochondrial pathway involves alterations of the mitochondrial potential, which triggers the production of ROS and/or mitochondrial membrane permeabilization (Biasi et al., 2009; G. Leonarduzzi et

al., 2007). Mostly endogenous COPs mediate apoptosis via the mitochondrial pathway, although a few recent studies have found evidence of actions on the caspase cascade as well (Sinéad Lordan and John, 2009).

The death receptor pathway is activated through the binding of cytokine ligands to receptors of the tumor necrosis factor (TNF) superfamily, such as Fas, lymphotoxin, TNF receptor (TNFR) or TNF-related apoptosis-inducing ligand (Sinéad Lordan and John, 2009). Both 7β-OH and 25-OH up-regulated Fas expression and induced apoptosis in vascular smooth cells when treated with oxidized LDL (oxLDL) (T. S. Lee & Chau, 2001). However, 7β-OH and 7-keto failed to induce the proapoptotic ligand TNF-α in human umbilical venous endothelial cells (HUVECs), but triggered apoptosis by activating the interleukin IL-1β secretion only after 24 h of incubation, suggesting a time-dependent increase after exposure (S. Lemaire et al., 1998). 7β-OH, 7-keto and 5,6β-epoxide also induced the release of the inflammatory cytokine IL-8 in U937 (human promonocytic leukemia cells), monocytes/macrophages, as much as THP-1 cells (Lemaire-Ewing et al., 2005; Y. Liu, Mattsson Hultén, & Wiklund, 1997). Several protein kinases involved in the upstream induction and downstream execution stages of apoptosis have shown modified activity after exposure to 7β-OH and 7-keto (Adamczyk, Scherrer, Kupferberg, Malviya, & Mersel, 1998; Berthier et al., 2005). The activity of protein kinase C – a key enzyme of the cell activation pathway (Moog et al., 1991) - is reduced by exposure of 7β-OH in neuronal cells (Sinéad Lordan and John, 2009), but not in macrophages (Luu, 1991; Moog et al., 1991), indicating a cell dependency in protein kinases' regulations.

More interesting is the potential dysregulation of mitochondrial control exerted by several COPs, since it is more directly involved with oxidative stress. 7β-OH, 7-keto, and 5,6β-epoxide have shown to induce loss of mitochondrial transmembrane potential

(Lemaire-Ewing et al., 2005), which is accompanied by release of cytochrome c in the cytosol (Gérard Lizard and Carole Miguet and Ginette Besséde and Serge Monier and Serge Gueldry and Dominique Neel and Philippe, 2000). Cytochrome c, an essential hemoprotein of the respiratory chain, has an intermediate role in apoptosis by triggering caspase 9, which in turn activates caspase 7 and 3 (Wolf and Green, 1999). The cytochrome c apoptotic pathway has been observed in several cell lines, including U937 (Gérard Lizard and Carole Miguet and Ginette Besséde and Serge Monier and Serge Gueldry and Dominique Neel and Philippe, 2000), MCF-7 and MCF-7/c3 cells (Prunet, Lemaire-Ewing, Ménétrier, Néel, & Lizard, 2005), upon exposure of 7-keto and 7β-OH. Regarding caspase activation, COPs activate both initiator and effector caspases, thus exerting activity at both up and downstream steps of the apoptotic process. Several reports have put 7-keto, 7β-OH and 25-OH in the frontline for activation of caspase 8 and 12 (initiator caspases), as well as caspase 3 (effector caspase) (T. S. Lee & Chau, 2001). Interestingly, several studies agree that the isomer 7α-OH has no effect on the caspase cascade (S. Lemaire et al., 1998; Lemaire-Ewing et al., 2005; Lordan, Mackrill, & O'Brien, 2009; Wolf & Green, 1999).

Other mechanisms of cytotoxicity have been reported, including altered transport of small molecules via alteration of plasma membrane fluidity and permeabilization, cell detachment, leakage of cell enzymes, as well as interference with DNA synthesis (*APPENDIX A – Table 2*). The mutagenic activities of COPs have been known since the early ’80s (Jusakul, Yongvanit, Loilome, Namwat, & Kuver, 2011; Sevanian & Peterson, 1984, 1986). The 5,6-epoxy isomers and the derived triol have demonstrated inhibition activity towards DNA synthesis in hamster V79 cells at micromolar concentrations (Sevanian & Peterson, 1984); similar results have been reported for the 7-keto, whose effect are synergic with the epoxides (Sevanian & Peterson, 1986). It is believed that the mutagenetic effect relates to

the ability of epoxides of generating ROS (Jusakul et al., 2011). Other potential pro-carcinogenic effects, including DNA fragmentation, have been observed in Caco-2 cells colon cancer by 7α-OH, 7β-OH and 5,6α-epoxy (Biasi et al., 2013; Roussi et al., 2005). In prostate cancer, 27-OH can induce DNA damage, regulate cyclooxygenase-2 expression, and stimulation of tumor cell migration (Cruz et al., 2010; Zarrouk et al., 2014), which is accompanied by proliferation, stimulation and increase of androgen receptor (AR) transcriptional activity (Raza et al., 2016).

### 1.2.5 COPs and Cell Survival Mechanisms

Concurrently with pro-apoptotic triggering, COPs have been lately associated with survival anti-apoptotic mechanisms (Beyza Vurusaner and Paola Gamba and Gabriella Testa and Simona Gargiulo and Gabriella Leonarduzzi and Giuseppe Poli and Huveyda, 2016); (Beyza Vurusaner and Gabriella Leonarduzzi and Paola Gamba and Giuseppe Poli and Huveyda, 2016). A study by Berthier and co-workers (Berthier et al., 2005) proved that 7-keto can delay the apoptotic effect induced by 7- keto itself at relatively high concentrations (100 μM). Indeed, 7-keto seemed to transiently induce the MAPK-Erk kinase-1 and 2 survival pathways (MEK 1/2 → ERK 1/2), inhibiting THP-1 human monocytes death by phosphorylating the Bcl-2 antagonist of cell death (BAD), which then delays mitochondrial damage (G. Leonarduzzi et al., 2010). A concentration-dependent behavior in U937 macrophages was also found for the side-chain enzymatic 27-OH, derived from CYP27A1 activity (A. Vejux & G. Lizard, 2009). At low concentration (2 μg/mL), 27-OH inactivates the PI3K/Akt survival cascade via phosphorylation of Thr308 residue in Akt, whereas at higher concentrations (40 μg/mL) rapidly triggers lysosomal-independent apoptosis (Valérie Riendeau and Christophe). An analogue concentration-dependent survival signaling was

observed for 7β-OH in HUVEC cells by activation of the MEK/ERK cascade (Trevisi et al., 2010).

## 1.3 DIETARY COPS: OCCURRENCE IN FOOD

Historically, the evaluation of food quality and safety during processing, preservation, and storage has relayed in targeted single response studies, evaluating quality aspects after a particular treatment (i.e., increase stability, improve texture, flavor, and digestibility), or mainly been focused on microbial reduction. On the other hand, lipid and cholesterol degradations are complex phenomena that can be addressed only by a multi-response analysis, which is hard to achieve considering the intrinsic difficulties of monitoring several molecules possessing different chemical nature. Although several reports have been published in the last decades (Baggio & Bragagnolo, 2006; Brzeska, Szymczyk, & Szterk, 2016; D. Derewiaka & Obiedzinski, 2010; I. Medina Meza & Barnaba, 2013; Vesa M Olkkonen et al., 2017; Sarantinos, 1993; Savage et al., 2002; Sieber, 2005; Zardetto, Barbanti, & Rosa, 2014), the information regarding COPs' content in foods is still incomplete. The limited data currently available has been obtained from studies performed in selected type of foods such as meat (Dorota Derewiaka & Obiedziński, 2009; Khan et al., 2015; Rey, López-Bote, & Buckley, 2004); (Andrea Serra and Giuseppe Conte and Alice Cappucci and Laura Casarosa and Marcello), eggs (Boselli, Velazco, Caboni, & Lercker, 2001; Mazalli & Bragagnolo, 2009; Tsai & Hudson, 1984) Tsai and Hudson, 2006), milk (Z. Liu, Rochfort, & Cocks, 2016; Rose-Sallin, 1995; Sieber, 2005), infant formula (Przygonski, Jelen, & Wasowicz, 2000; Romeu-Nadal, Chavez-Servin, Castellote, Rivero, & Lopez-Sabater, 2007) and few others. It is imperative to provide a database of COPs concentrations for the

major food products consumed by US population, with data obtained from real cooking procedures and by different population groups.

### 1.3.1 Food Processing Triggers COPs' Formation in Foods

Traditional food processing methods, such as drying, frying, steaming, and canning, involve heat treatment of the food matrix. It is well known for several macronutrients, including amino acids, carbohydrates as well as lipids, that heat conveys sufficient energy to trigger autoxidation, causing the formation of several degradation compounds. For example, acrylamide and malondialdehyde are end-products of Maillard reaction and fatty acids lipid peroxidation, respectively. For their toxicity towards humans and their occurrence in foods, both have been classified as hazardous substances, and their risk exposure in humans has been constantly monitored in both United States and Europe (Dorne, Bordajandi, Amzal, Ferrari, & Verger, 2009). Similarly, cholesterol oxidation gives rise to several degradation products whose toxicity has gathered the attention of the scientific community worldwide. Given the susceptibility of the C5- C6 double bond to a radical attack, heat triggers the cholesterol oxidation at temperatures as low as 100 °C (I. G. Medina Meza, Rodriguez-Estrada, Lercker, Soto-Rodríguez, & Garcia, 2011; J.-S. Min et al., 2015). These temperatures are easily achievable with common processing and food preparation techniques. Industrial processing (i.e., cooking, pasteurizing, canning, etc.) occurs at variable times and temperatures depending on the type of process and food. Effective canning of meats for example, takes place at 121 °C. Industrial frying takes place between 163 and 188 °C, depending on the type of meat; whereas in spray drying, a technique used to produce powders from a liquid, often used in the manufacturing of baby formulas, temperatures between 200 and 400 °C are used (Meister, Aebischer, Vikas, Eyer, & De

Pasquale, 2000; Verardo, Riciputi, Messia, Marconi, & Caboni, 2016). As a matter of facts, it has been found that several cooking methods considerably increase the formation of COPs in meats (Broncano, Petron, Parra, & Timon, 2009; Mar Roldan and Teresa Antequera and Monica Armenteros and Jorge, 2014). Microwave heating (Herzallah, 2005), pan roasting, oven grilling (Khan et al., 2015), oil-frying (D. Derewiaka & Obiedzinski, 2010) and other forms of cooking (Nielsen, 1996b) all affect the production of COPs. According to the US Department of Health and Human Services, meat and poultry should be roasted at a minimum of 162 °C (FoodSafety.gov, 2020b). Even proper roasting temperatures are more than sufficient to produce COPs (Shozen, 1995). Additionally, a variety of non-traditional methods of processing, including infrared heating, microwave heating, ohmic heating, high pressure processing, ionizing radiation, pulsed electric field and ultrasound are believed to be less harsh on meat products (Avsaroglu, Buzrul, Alpas, Akcelik, & Bozoglu, 2006); (Ilce Gabriela Medina-Meza and Carlo Barnaba and Gustavo, 2014; Medina-Meza et al., 2014). Compared to traditional technologies, these novel thermal and nonthermal technologies reduce/eliminate temperature exposure or decrease treatment time, but do not eliminate oxidation triggering factors all together (Herzallah, 2005; I. Medina-Meza & C. Barnaba, 2013; I. G. Medina-Meza, Barnaba, & Barbosa-Cánovas, 2014; Medina-Meza et al., 2014). However, we are far from a conclusive evidence that those novel technologies are reliable alternatives to lipid and cholesterol degradation (Ilce Gabriela Medina-Meza and Carlo Barnaba and Gustavo, 2014; Medina-Meza et al., 2014). Prepared meals – like ready-to-eat foods – also pose a significant risk (Ubhayasekera, Kochhar, & Dutta, 2006). These meals usually undergo several processing stages, from heating to pasteurization temperatures, followed by freezing, as part of the storage process before distribution; once stored for days, ready-to-eat meals finally make it home where they are re-heated or microwaved. It has

been found that refrigeration as well as re-heating increase the formation of COPs (J. S. Min et al., 2016).

### 1.3.2 Baby Foods

Exposure to food-derived toxic compounds can be critical in sensitive populations, especially infants and children. Powdered milk is a known source of COPs, although the reported amounts differ according to the milk process (Dionisi et al., 1998; Gabriella Leonarduzzi et al., 2005; Pickett-Bernard, 2006; Przygonski et al., 2000; Romeu-Nadal et al., 2007; Scopesi et al., 2002). Powdered milk is manufactured using the wet blending-spray drying process in cow milk. This process begins with milk pasteurization to decrease the pathogenic bacteria. The milk is then evaporated at 77 °C and then sent to the spray drying unit. This unit pre-heats the product up to 93 °C; the product is then pumped in the spray dryer at 138–204°C. This process provides the proper conditions for triggering hydroperoxide formation leading lipid and protein oxidation, among other reactions (Damjanovic-Desic, S., & Birlouez-Aragon 2011; Sieber, 2005). McCluskey and co-workers found up to 60 ppm COPs in skim powdered milk (McCluskey, 1993), whereas Scopesi et al. (2002) demonstrated that the content in 7-keto is in baby formula is 5-fold higher than human breast milk. Infant formula plays an important role in infant growth and health; according to a report from the US Institute of Medicine, it may be the only source of nutrition for many infants during the first 4–6 months of life (2006). Around 2.7 million of infants by the age of three months rely on it for some portion of their nutrition (Martin, Ling, & Blackburn, 2016). Infant formulations are enriched with several additives, including essential polyunsaturated fatty acids (PUFA), phytosterols, vitamins, which may play a synergistic role in the oxidation promotion of COPs. PUFAs are highly susceptible to

oxidation, and hence favored the cholesterol oxidation as well (Barnaba, Rodriguez-Estrada, Lercker, Sergio Garcia, & Medina-Meza, 2016; Barriuso, Ansorena, Poyato, & Astiasarán, 2015; Barriuso, Mariutti, Ansorena, Astiasarán, & Bragagnolo, 2016). Among them, docosahexaenoic acid (DHA), and arachidonic acid (ARA) are the most common fatty acid of the PUFA family used in enriched milks, they are critical for the brain and retina development, and therefore have an influence upon visual acuity and learning abilities (Romeu-Nadal et al., 2007). As infants progress from formula to solid foods (i.e., meat, eggs, cheese, etc.), their exposure to COPs will potentially increase and affect their metabolism (Sander, Addis, Park, & Smith, 1989). Zunin et al. (2006) found considerable amounts of 7-keto and triol in meat-based homogenates (2.3 g and 0.7 g per serving respectively), which was dramatically increased when vegetable oil was added to the formulations.

### 1.3.3 Food Chain: Packaging and Storage

Food products are packaged, shipped, and stored as part of their processing before retail. Packaging and storage methods can also affect the formation of oxidative products. It has also been shown that storage time can increase cholesterol oxidation (Du & Ahn, 2000; Mazalli & Bragagnolo, 2007; Nielsen, 1996a; Petrón, 2003; Pie, 1991; Tarvainen, Nuora, Quirin, Kallio, & Yang, 2015; Vore, 1988). The content of COPs has been reported to increase by six times after 2 weeks of storage at 4 °C, being higher in cooked meat than in raw food. Therefore, vacuum storage of cooked meat products has been suggested as an alternative to reduce their formation (J. S. Min et al., 2016). Reduction in surface area of food exposed to the atmosphere and the amount of light absorbed by the food, as well as the addition of a surface spray and light reflecting or absorbing packaging can all help reduce the formation of COPs (Khan et al., 2015; Li, Cherian, Ahn, Hardin, & Sim, 1996;

Mariutti & Bragagnolo, 2017; I. G. Medina-Meza et al., 2014; Medina-Meza et al., 2014; J.-S. Min et al., 2015; Overholt et al., 2016; Savage et al., 2002). A decrease in time from farm to table could also considerably decrease their accumulation. Changes in food consumption habits can represent a challenge in terms of establishing exposure to toxic compounds derived from diet. In the United States, the consumption of ready to eat (RTE) and "deli" food products have increased in the last decade. Sandwiches, hot dogs, hamburgers, wrap or "subs" are some of the most RTE popular products. Frozen food products are usually thawed and microwaved before consumption. Heat produced during microwaving of food have shown to accelerate the chemical oxidation (Aziz, Mahrous, & Youssef, 2002; Picouet, Fernandez, Serra, Sunol, & Arnau, 2007; Savage et al., 2002; Yarmand & Homayouni, 2009), compared to traditional cooking. Home meal preparation may affect not only cholesterol and lipids, but also protein fraction and its derivatives enhancing the probability of the food matrix to undergo several oxidative transformations. Logistics between farmers, processors, shipping companies and retail locations could be an important step in reducing the storage time of animal food products. However, although there is some knowledge on the relationship between packaging and storage conditions, there is still a need of experimental data regarding the effect of storage time and other storage conditions (as well as other food cooking methods and conditions) on COPs' content. As previously explained, these thermal changes vary among the food matrix, and the cooking methods and conditions; hence, COPs' formation becomes not only directly related to the food matrix composition, but also in the processing method, packing and storage conditions.

## 1.4 COPS: A FOOD TOXICOLOGICAL TARGET?

### 1.4.1 Fate of the COPs: Risk and Exposure Assessment

Humans are exposed to a variety of substances from multiples exposure routes (i.e., inhalation, contact, ingestion) and sources (i.e., air, food, soil, water) (Dorne et al., 2009). Risk is the probability of an adverse effect on man occurring because of a given exposure to a chemical or mixture, while hazard is the inherent capacity of a chemical or mixture to cause adverse effects in man under conditions of exposure (Hanlon, Brorby, & Krishan, 2016). Food toxicology has emerged as a critical topic for both the scientific community and governments, leading to the formation of different projects, like the Total Diet Study (TDS) led by the Food and Drug Administration (FDA, 2014) in the United States. The project started on 1961 and aims to systematically monitoring several contaminants and nutrients present in the average U.S. diet. Unfortunately, even though special attention has been given to the analysis of the American diet, there is no specific study on the risk exposure of COPs. Currently, there are no federal regulations for food processing and storage conditions considering the content of COPs and the consequent human risk exposure, even considering the broad body of evidence demonstrating the direct relationship between COPs and several chronical diseases (Del Rio et al., 2013; Mukhopadhyay et al., 2017). This information is essential to pursue more detailed studies on the relationship between COPs' intake and toxicological effects. The TDS is monitoring about 800 compounds in the US diet during 2014 –2017 (FDA, 2014). Selected pesticides, herbicides, radionuclides, nutrients, and toxic elements are tracked year by year through 4 market basket of food products. The food database used in the TDS can potentially be a good starting point to perform a systematic monitoring of the occurrence of COPs and to estimate the annual dietary intakes in the U.S. population. Gaining a deep understanding of how and from what sources humans consume

cholesterol oxidative products could provide information to help reduce consumption. A decrease in COPs' consumption would lead a decrease in the side effects of COPs related chronic diseases.

From studies published decades ago, the toxicity and the potential hazard activity of COPs have been demonstrated; however, being an "ensemble" of molecules rather than a single compound, performing an assessment of COPs exposure is challenging. Ideally, occurrence data with concentrations and frequency should be available in exhaustive, consistent lists. In practice, these conditions rarely met. The most common information on food consumption is derived from dietary surveys, usually conducted on a representative sample of individuals. Some surveys are based on food-frequency, dietary-history questionnaires, or household purchases without cover longer periods of times in term of individual dietary consumption (Dorne et al., 2009). Geographical and cultural differences can influence exposure levels of COPs. Because of their diet rich in fats and meat products, the U.S. population may be exposed to high cholesterol diet, and therefore its oxidative products, at a very early age. In addition, individuals, and particular subpopulations (i.e., infants, children, and elder people) may be exposed or respond differently to these chemicals (Cote et al., 2016). Datasets regarding exposure and effects are needed to evaluate the dose-response (toxicological assessment) and further risk characterization of COPs. For a priori hazard assessment a dietary exposure model should be performed. A combination of exposure (total dietary intake) and kinetic modelling (dose-depending activity) could give the initial hints in the estimation of human exposure to COPs. Informing industry members, stakeholders, and federal agencies of the dietary intake of COPs and their negative health effects is crucial to take the proper actions in reduce its production during the whole food chain.

### 1.4.2 COPs as Biomarkers of Food Safety

Several omics (i.e., metabolomics, lipidomics, and nutrigenomics, among others) have been already applied to characterize postharvest metabolite heterogeneity of fruits (Romina Pedreschi and Pablo Muñoz and Paula Robledo and Cecilia Becerra and Bruno, 2014), food toxins (Jasminka Giacometti and Alena Buretić Tomljanović and Djuro, 2013), and nutritional components (Vergères, 2013) for studying biomarkers (Bordoni & Capozzi, 2015), as well as metabolic pathways (Jain, Dürr, & Ayyalusamy, 2015). In the food area, foodomics and metabolomics profiling have been mainly focused on food quality, safety, and nutrition (Sooah Kim and Jungyeon Kim and Eun Ju Yun and Kyoung Heon, 2016); nonetheless the application of omics to evaluate and trace effects of processing as fingerprint of the process operations themselves is scarcely used.

The assessment of dietary level of cholesterol and its derivatives combines dietary consumption data with occurrence. Cholesterol information should be found in the nutritional label that the FDA requests for the food's products derivatives. This information may be insufficient for a consistent and systematic monitoring of COPs content in food, to support a risk assessment study. Challenges in cholesterol and COPs' quantitation has been reduced by applying omics approaches to the field, i.e., cholesterolomics, a field that is dedicated to the extraction, isolation, and quantification of the cholesterome in food, cells, tissues, organs and biofluids ((Griffiths et al., 2017). Chromatographic techniques couple with single (GC-MS) or triple quadrupole (LC-MS/MS) have been enhancing COPs' detection and quantification (Chiu, 2018; Helmschrodt et al., 2013). 7-Keto has been largely detected and quantified in both model and food systems (Rodriguez-Estrada, Garcia-Llatas, & Lagarda, 2014). During thermal-induced oxidation, hydroperoxides in C-7 are mainly generated, thus 7-keto is one of the most representative COPs in food systems ranging from

30% to 70% of the total content of COPs. This has suggested that 7-keto can be the most reliable biomarker of cholesterol oxidation due high temperature processing of foods (Rodriguez-Estrada et al., 2014). However, other processing such as ultra-high temperature (UHT) pasteurization can also generate sidechain COPs (Pikul et al., 2013), including the demonstrated cytotoxic 25-OH. It is still unclear if COPs' composition can reflect specific processing technologies, as some early studies have demonstrated. For example, gamma radiation triggers the specific oxidation of cholesterol in the positions C5 and C6 (Maerker & Jones, 1993a; Ilce Gabriela Medina-Meza et al., 2012). However, more mechanistic, and quantitative studies for a suitable estimation of COPs in food products, and a harmonization of methodologies for their determination is still needed.

## 1.5 PARADIGM SHIFTS AND FUTURE CHALLENGES

### 1.5.1 Oxidative Mechanisms: Holistic Approach

Many conditions are responsible of trigging/inhibiting the formation of cholesterol oxidative products in food preparations, as reviewed in APPENDIX A, Tables 3–7. Given the discussed complexity on the oxidative phenomena in the food matrix, any predictive model that would aim to considers all chemical and biochemical parameters, as well as intrinsic and extrinsic factors, is enormously challenging. Analytical strategy can potentially "dissect" the oxidative process considering a few factors at the time. Biomimetics and model systems have been widely used to reproduce several aspects of the cell or tissues outside a living system and are useful to represent a real-world phenomenon. These experimental settings may help to understand the biochemistry associated to the formation of COPs. Models for effects of heating on cholesterol oxidative product formation (Chien et al., 1998) and for high pressure processing's effects on cholesterol oxidative product formation (Ilce Gabriela

Medina-Meza & Barbosa-Cánovas, 2015; Medina-Meza et al., 2014) have been already developed. Further model systems that can account for raw material composition and heterogeneity, processing and storage time and conditions, presence/absence of antioxidants: together, this information could also be very beneficial in assessing COPs' risk exposure. On the other hand, cell biomimetics and animal studies can effectively provide information regarding COPs physiological absorption. Model systems not only can help to predict the concentrations of cholesterol oxidative products in certain foods, tissues, cell lines and body fluids but also can be used to further understand human exposure to cholesterol oxides (Egeghy et al., 2016). In our opinion, a holistic approach in the cholesterol and lipid oxidation fields should include targeted and high throughput analytical techniques along with higher order statistical modelling. The application of "processomics" (Tara Grauwet and Liesbeth Vervoort and Ines Colle and Ann and Marc, 2014) for fingerprinting of food/biological markers and exposure assessment will potentially address the following questions: What is the association between dietary COPs and the major chronic diseases? Are COPs just food biomarkers? Given the power of holistic and as consequently translational approaches in the cholesterolome development, the improvement of analytical resolution and sensitivity, markers fingerprinting, and modelling is highly encouraged.

## 1.6 CONCLUSIONS

COPs are formed both chemically and enzymatically in foods and the human body. More holistic research is required to assess the contribution of dietary and endogenous sources to the total COPs levels found in animals and humans. The need of a systematic review of the toxicology activities of COPs and the estimation of daily intake of COPs is mandatory, to understand the connection between diet, food manufacturing and

epidemiology, which will be a “key component” in this puzzle. Additionally, the creation of a culture of prevention towards cholesterol and lipid oxidation in foods should be encouraged, together with the preservation of intrinsic natural antioxidants, as a strategy that can dampen the formation and accumulation of COPs in foods.

# CHAPTER 2 : PHYTOSTEROLS AND THEIR OXIDATIVE PRODUCTS IN INFANT FORMULA

The content of this chapter has been previously published in the Journal of Food Process Engineering and is an equal contribution between authors: Alice Kilvington and Lisaura Maldonado-Pereira. DOI: 10.1111/jfpe.13151

## 2.1 INTRODUCTION

Phytosterols also known as plant sterols (PS), are natural compounds which are members of the triterpene family. The triterpene group includes more than 4,000 compounds and over 100 of those are phytosterols. In food matrices, PS have been chemically characterized and quantified for over three decades (Moreau, Whitaker, & Hicks, 2002). PS are 28- or 29-carbon alcohols with a steroid nucleus, a 3β-hydroxyl group, and a double bond in the C5–C6 position. Compared to cholesterol, PS contain an extra methyl group, ethyl group, or double bond with a side chain of 9–10 carbon atoms in length, instead of a C8 cholesterol side chain. In plant cells, PS are primarily encountered in the plasma membrane, specifically in the outer membrane of mitochondria and the endoplasmic reticulum. They play an important role as a structural molecule, providing rigidity to the cell membrane by promoting an increase in the sterol/phospholipid ratio that is associated with membrane stiffness (Alemany, Barbera, Alegría, & Laparra, 2014; Comunian & Favaro-Trindade, 2016; Moreau et al., 2002). PS are components of all foods of vegetable origin and are known for their beneficial property in health of lowering serum total cholesterol concentration, as well as the low-density lipoproteins (LDLs) concentration (Lagarda, García-Llatas, & Farré, 2006). Therefore, for over the past 15 years, PS have been incorporated into several functional foods as a supplement (García-Llatas & Rodríguez-Estrada, 2011). The structure of the most common phytosterols is shown in Figure 2-1. The

most common phytosterols found in foods are β-sitosterol, campesterol, stigmasterol, and Δ5-avenasterol. β-sitosterol is the most abundant and has a proportion of total sterols content of 60–70% (Berger, Jones, & Abumweis, 2004; Verhé, Verleyen, Hoed, & Greyt, 2005).

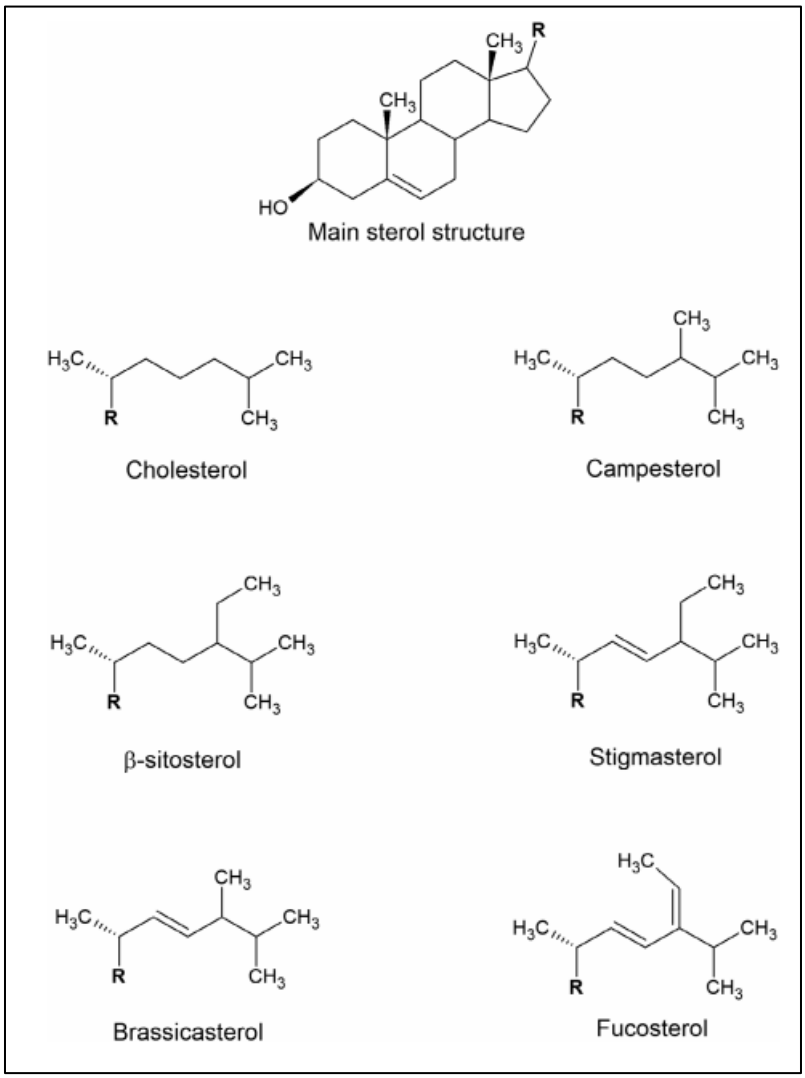


***Figure 2-1:*** *Structures of the most common phytosterols obtained from Kilvington et al. 2019.*

## 2.2 PHYTOSTEROLS FOOD SOURCES

Vegetable oils are the major sources of PS in foods. Palm, sunflower, corn, coconut, rapeseed, and soybean oils have the highest content of total sterols; for example, rapeseed oil has a PS content ranging from 646 to 808 mg/100 g in fresh weight (FW); oil-based products like margarines have a content ranging from 130 to 540 mg/100 g FW (Normén, Ellegård, Brants, Dutta, & Andersson, 2007). Other food items such as cereals (corn, rye, wheat, barley, and oats) range from 80–90 mg/100 g FW (Ryan, Galvin, O'Connor, Maguire, & O'Brien, 2007). Additionally, cereals derivatives and nuts are great source of PS, containing the highest quantities of phytosterols compared with other foods.

Another important source of PS are fruits and berries, which are popular and highly consumed due their many beneficial nutritional properties such as their antioxidant capacity, with content ranging from 6 to 75 mg/100 g FW (Piironen, Toivo, & Lampi, 2000). PS dietary uptake varies greatly depending on the region, country and/or culture. In Northern Europe, up to 40% of dietary PS derive from cereals and cereal-based foods. However, in countries having greater availability of fresh fruits the main contributor to PS in the diet are fruits and vegetables, contributing as much as 35% of dietary PS, as a study performed in Uruguay showed (Lea, Hepburn, Wolfreys, & Baldrick, 2004; Piironen et al., 2000). Databases of almost every type of food have been developed over the years, and even country specific food items have been analyzed such as the ones reported by (Normén et al., 2007) which refers to the number of PS found in spreads, oils, seeds, and various other fatty foods typically consumed in Sweden and the Netherlands. PS content in foods has been summarized in Table 2-1. Animal origin food items are included because of the addition of vegetable oils or ingredients as part of different food processing techniques.

However, the previously mentioned variability of PS concentration in animal origin food items, based on the plant's origin of the additive is strongly noticeable between food items. Some differences are due to the supplementation of plant-based oils or even because of the various diets of cows, pigs, chickens, and other animals typically consumed by humans, which contain plant products.

## 2.3 HEALTH BENEFITS OF PHYTOSTEROLS

The chemical similarity of PS to cholesterol has played an important role in the study and analysis of the physiological aspects of PS. Most of its chemical properties have been studied based on the information known for cholesterol (Maldonado-Pereira, Schweiss, Barnaba, & Medina-Meza, 2018). Absorption of PS in humans is low compared to cholesterol, ranging from 2 to 5% of total intake, versus 60%, respectively, (Alemany et al., 2014; Mellies et al., 1976) performed a study on infants and children to see the effects of dietary phytosterols. They found that infants fed with infant formulas enriched with phytosterols had blood plasma levels of campesterol and β-sitosterol that were three to five times higher than infants fed with breast or cow's milk. At the time when this initial quantitative assessment was performed, little was known about the potential biological implications of phytosterols in infants (Mellies et al., 1976; R. Ostlund, Racette, Okeke, & Stenson, 2002). Due to their structural similarity to cholesterol, PS were first and foremost studied for their cholesterol absorption inhibition properties. They are well known for their ability to reduce cholesterol absorption (Nestel, Cehun, Pomeroy, Abbey, & Weldon, 2001; R. Ostlund et al., 2002; R. Ostlund, Racette, & Stenson, 2003; R. E. J. Ostlund, 2002), which is reflected in a reduced cholesterol plasma concentration (Law, 2000; Nguyen, 1999; Piironen et al., 2000; Pinedo et al., 2007); lipoprotein oxidation reduction anti-inflammatory

properties (Gabay, Lamacchia, & Palmer, 2010), anti-cancer properties, and apoptosis induction, positive regulation on testosterone metabolism (Awad, Hernandez, Fink, & Mendel, 1997), cancer cell proliferation reduction by angiogenesis inhibition (Lea et al., 2004; Shahzad et al., 2017), and tumor growth reduction (Danesi et al., 2011; Llaverias et al., 2013).

***Table 2-1:*** *Summary table of the phytosterol content in oils and foodstuff published in Kilvington el at. 2019*

| **Oils** | | | | |
|---|---|---|---|---|
| **Food** | **Processing conditions** | **Phytosterol** | **Concentration** | **Reference** |
| Sunflower | N/A | Sitosterol | 233 ± 4 mg/100 g | Lin et al. (2017) |
| | | Campesterol | 34 ± 0 mg/100 g | |
| | | Stigmasterol | 32 ± 0 mg/100 g | |
| | | Sitostanol | 12 ± 0 mg/100 g | |
| Rapeseed | N/A | Sitosterol | 378 ± 13 mg/100 g | |
| | | Campesterol | 290 ± 10 mg/100 g | |
| | | Brassicasterol | 83 ± 2 mg/100 g | |
| | | Sitostanol | 59 ± 1 mg/100 g | |
| **Dairy** | | | | |
| Milk | N/A | Total phytosterols | 0.156 ± 0.00 g/100 g | Srigley and Haile (2015) |
| Eggs | N/A | Total phytosterols | 20.7 mg sterols/100 g ingredient | Menéndez-Carreño, Knol, and Janssen (2016) |
| **Meat and Poultry** | | | | |
| Meat (steak, roast beef, stew, chicken, pork, and minced meat) | N/A | Total phytosterols | 3.47–14.9 mg sterols/100 g ingredient | Menéndez-Carreño et al. (2016) |
| **Seafood** | | | | |
| Fish (salmon, shallow-fried cod, microwaved cod, and fish sticks) | N/A | Total phytosterols | 2.01–51.9 mg sterols/100 g ingredient | Menéndez-Carreño et al. (2016) |

*Table 2-1 (cont'd)*

| Others | | | | |
|---|---|---|---|---|
| margarine | - Control<br>- Storage (18 weeks @ 4ºC)<br>- Storage (18 weeks @ 20ºC) | Brassicasterol | - 0.82 ± 0.18 g/100 g spread (control)<br>- 0.71 ± 0.06 g/100 g spread (18 weeks @ 4ºC)<br>- 0.58 ± 0.05 g/100 g spread (18 weeks @ 20ºC) | Rudzinska, Przybylski, and Wąsowicz, (2014) |
| | | Campesterol | - 2.95 ± 0.23 g/100 g spread (control)<br>- 2.12 ± 0.18 g/100 g spread (18 weeks<br>- @ 4ºC)<br>- 2.04 ± 0.18 g/100 g spread (18 weeks<br>- @ 20ºC) | |
| Light mayonnaise | N/A | Total phytosterols | 0.686 ± 0.01 g/100 g | Srigley and Haile (2015) |
| Spread/margarine 1 | | | 0.788 ± 0.01 g/100 g | |
| Spread/margarine-2 | | | 0.753 ± 0.01 g/100 g | |
| Spread/margarine 3 | | | 2.88 ± 0.02 g/100 g | |
| Spread/margarine 4 | | | 3.82 ± 0.04 g/100 g | |
| Orange juice | | | 0.423 ± 0.01 g/100 g | |
| Protein shake 1 | | | 0.529 ± 0.02 g/100 g | |
| Protein shake 2 | | | 4.86 ± 0.60 g/100 g | |
| Instant coffee | | | 6.96 ± 0.69 g/100 g | |
| Dietary chew 1 | | | 7.73 ± 0.11 g/100 g | |
| Dietary chew 2 | | | 9.61 ± 0.09 g/100 g | |

The National Cholesterol Education Program recommends adding 2 g/day of phytosterols to the diet to reduce LDL cholesterol concentrations and coronary heart disease risk. In 2000, the FDA issued an interim rule allowing the claim that plant stanyl and steryl esters-containing foods reduce the risk of coronary heart disease, because their demonstrated cholesterol-lowering effect (Golley & Hendrie, 2014; Moreau et al., 2002). Moreover, PS success in health has been proved by the development of different patents and commercial PS products currently being marketed worldwide (García-Llatas & Rodríguez-Estrada, 2011).

## 2.4 PHYTOSTEROLS OXIDATION

PS are particularly susceptible to oxidation due to their surface activation property, by exposure to UV light, high by 2–5%, increasing the oxidation as consequence. The presence of heat, light, metal contaminants catalyze radical-mediated oxidation at the double bond, starting an autocatalytic oxidative chain reaction. Similarly, to general lipid oxidation processes, PS oxidation evolves through three main steps: the initiation corresponding to the generation of highly reactive radical species, the propagation of radical species via autocatalysis, and the termination of the reactions with consequent formation of thermodynamically stable compounds (Johnson & Decker, 2015). In foods, free radicals can be generated by photosensitization mediated by either chlorophyll (vegetable matrices; (I. G. Medina-Meza et al., 2014) or heme (animal matrices), which leads to the formation of singlet oxygen, or by reacting with metals (Boatright & Crum, 2016). Oxidation can also occur by pre-existing reactive oxygen species (ROS) and oxidative enzymes such as cytochrome P450, superoxides, and peroxidases naturally present in vegetable matrices (Ryan, McCarthy, Maguire, & O'Brien, 2009). The oxidation of PS in food follows similar chemical pathways to cholesterol oxidation, and the products of PS oxidation are known as phytosterol oxidation products (POPs; (I. G. Medina-Meza & C. Barnaba, 2013). PS major oxidation pathways are shown in Figure 2-2. Primary products of oxidation are the allylic 7-hydroperoxides, which are further oxidized to the corresponding 7α- and 7β-hydroxy by epimerization. Hydroxyls can be further oxidized to the chemically stable 7-keto compounds, as well as the highly reactive 5,6α- and 5,6β-epoxy compounds. The epoxides generally are hydrolyzed to form the 3,5,6β-triols (Barriuso, Otaegui-Arrazola, Menéndez-Carreño, Astiasarán, & Ansorena, 2012; Cercaci, Rodriguez-Estrada, Lercker, & Decker, 2007;

González-Larena et al., 2011; Lin et al., 2018; Lin et al., 2017; O'Callaghan, McCarthy, & O'Brien, 2014).

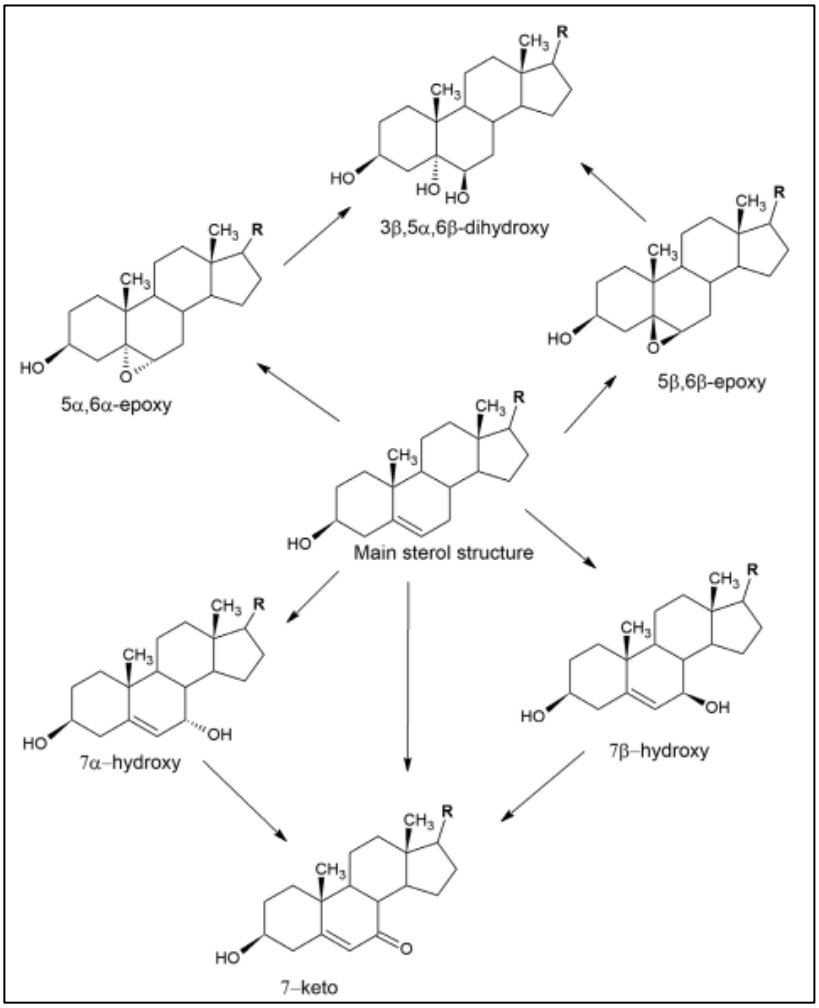


***Figure 2-2:*** *Figure published in Kilvington et al. 2019 describing the general phytosterol oxidation pathway where R denotes a specific phytosterol side chain.*

Main factors promoting POPs formation in foods are: (a) processing temperature and time, (b) storage temperature: affects only when temperature is over 34ºC, (c) sterols structure, (d) esterification, (e) degree of saturation, and (f) food matrix (Danesi et al., 2011). The concentration of POPs in various food products is shown in Appendix B – Table 2. POPs formation increases proportionally to temperature and time; POPs formation is also

affected by water content and oil drop size in the food matrix (Cercaci et al., 2007; McClements, Decker, & Weiss, 2007). Moreover, when producing rapeseed oil industrially, it was found that the refined oil had over double the POP content (Rudzinska, M., Uchman, & Wasowicz, 2005).

## 2.5 POPS BIOLOGICAL TOXICITY

Few studies have given some insight about POPs and their biological activity both in humans and animal models. Studies performed in animals have demonstrated that POPs can be absorbed at a higher rate compared to phytosterols (A. Grandgirard, Sergiel, Nour, Demaison-Meloche, & Giniès, 1999; Meynier, Andre, Lherminier, Grandgirard, & Demaison, 2005). POPs distribution and accumulation in different animal tissues (aorta, heart, kidneys, liver) revealed the highest concentration of hydroxyl derivatives in the liver (Bang, Arakawa, Takada, Sato, & Imaizumi, 2008; Liang et al., 2011; Tomoyori et al., 2004), but triols were also found in the liver, kidney, and the heart lipid fraction. For example, lymphatic recoveries for campesterol oxides (16%) and sitosterol oxides (9%) were higher than for campesterol (6%) and β-sitosterol (2%); (A. Grandgirard, Demaison-Meloche, Cordelet, & Demaison, 2004; A. Grandgirard, Martine, et al., 2004). These trends are similar to the absorption of non-oxidized phytosterols, implying that increasing the side chain length of either the PS or POPs, decreased their absorption and that the type of oxidation relates to the degree of absorption (Ryan et al., 2007).

POPs may be related to the inflammation processes, dyslipidemia, atherosclerosis, apoptosis, and cell toxicity (Alemany et al., 2014; Y. Hu et al., 2018; P. Zunin, Calcagno, & Evangelisti, 1998). Adcox, Boyd, Oehrl, Allen, and Fenner (2001) demonstrated that POPs affect protein synthesis and damage the cell membrane, while measuring total protein

content and LDL leakage (Adcox, Boyd, Oehrl, Allen, & Fenner, 2001). Recently, it has been hypothesized that due to the structural similarity between POPs and cholesterol oxidation products (COPs), POPs could potentially contribute to the onset of metabolic and neurologic diseases by an irreversible accumulation in the central nervous system. A recent study discovered that sitosterol and 7β-hydroxysitosterol can pass through the blood brain barrier (Schött et al., 2017; Schött et al., 2015). Maguire, Konoplyannikov, Ford, Maguire, and O'brien (2003) reported that thermally oxidized derivatives of β-sitosterol have shown similar patterns of toxicity towards a human monocytic cell line (U937) as the cholesterol-derivative 7β-hydroxycholesterol (30 μM), although higher concentrations of the POPs mixture (120 μM) was required. Studies with several cell lines that is, human monocytic (U937), colonic adenocarcinoma (Caco-2) and hepatoma (HepG2) cells, found that 7βhydroxy, 7-keto and triol derivatives of β-sitosterol were moderately cytotoxic (at 60 and 120 μM) to all three cell lines; the mode of cell death was apoptosis in the U937 cell line and necrosis in the Caco-2 and HepG2 cells (Ryan et al., 2007).

## 2.6 INFANT FORMULATION: A CRITICAL NEED

The United States Food and Drug Administration (FDA) defines Infant formula (IF) as “a food which purports to be or is represented for special dietary use solely as a food for infants by reason of its simulation of human milk or its suitability as a complete or partial substitute for human milk” (FDA, 2016). IF provides all nutrients and sustenance for the growth and development of infants when breast feeding is not an option for physiological or medical reasons (Green Corkins & Shurley, 2016; Su et al., 2017; Vandenplas, Zakharova, & Dmitrieva, 2015).

Different health issues, such as allergies and metabolic disorders, that infants could develop during their first months of life, are targeted in the production of numerous types of formulas that provide the appropriate nutrition for every child, adding or removing certain components of the infant formula based on the specific need of the infant (Maldonado, Gil, Narbona, & Molina, 1998). In the United States common types of infant formula are cow's milk-based, soy-based, lactose-reduced, or partially hydrolyzed, and specialty. These special formulas are developed for infants who have certain conditions, like protein sensitivity, acid reflux, pre-term, phenylketonuria, and so on. (Rossen, Simon, & Herrick, 2016). Since the infant's nutrition plays a crucial role in development both short and long term (M. Lemaire, Le Huërou-Luron, & Blat, 2018), this is the main reason why the FDA issued a Code of Federal Regulations (Title 21, Chapter I, Subchapter B, Part 107) where the requirements are outlined for the composition of IF. As it is stated by the FDA, for each 100-cal serving there must be 300 mg of Linoleic acid and 1.8–4.5 g of protein per serving ("Code of Federal Regulations", 2019). In addition, IF contains around 20% fat, which comes from vegetable oils like sunflower, palm, soy, or coconut oils (5, 8, 7, and 5%, respectively; (S. Damjanovic-Desic & Birlouez-Aragon, 2011; Hamdan, Claumarchirant, Garcia-Llatas, Alegría, & Lagarda, 2017; Hamdan, Sanchez-Siles, Garcia-Llatas, & Lagarda, 2018; Rajasekaran & Kalaivani, 2013). PS content in vegetables oils normally added to IF can be seen in Figure 2-3. Sterols content in human milk differ from IF's sterols content. The animal sterols, including cholesterol, range from 12.0 to 16.6 mg/100 mL and 0.4–5.47 mg/100 mL in human milk and IF, respectively. The phytosterols content is 0.02 mg/100 mL in human milk versus 2.45–5.07 mg/100 mL in IF, where the main phytosterols are β-sitosterol and stigmasterol (Hamdan et al., 2017). IFs are manufactured to mimic the human milk as close

as possible, but many differences remain, bringing up a nutritional concern because of its effect on the baby's health.

**Phytosterols concentrations in infant formula**

| Type | Phytosterol | Concentration | Reference |
|---|---|---|---|
| Powdered | Brassicasterol | 2.81 ± 0.08 mg/L | Hamdan et al. (2017) |
| | Stigmasterol | 5.04 ± 0.36 mg/L | |
| | β-sitosterol | 30.28 ± 0.23 mg/L | |
| | Campesterol | 13.80 ± 0.17 mg/L | |
| | Sitostanol | 0.98 ± 0.12 mg/L | |
| | Brassicasterol | 0.39 ± 0.01 mg/100 mL of reconstituted IF | Hamdan et al. (2018) |
| | Campesterol | 2.07 ± 0.02 mg/100 mL of reconstituted IF | |
| | Stigmasterol | 0.34 ± 0.06 mg/100 mL of reconstituted IF | |
| | β-sitosterol | 5.72 ± 0.48 mg/100 mL of reconstituted IF | |
| | Sitosterol | 350–1,480 mg/g lipid | Zunin et al. (1998) |
| Liquid | Brassicasterol | 0.18 ± 0.02 mg/100 g | García-Llatas et al. (2008) |
| | Campesterol | 1.18 ± 0.09 mg/100 g | |
| | Stigmasterol | 0.37 ± 0.05 mg/100 g | |
| | β-sitosterol | 3.47 ± 0.37 mg/100 g | |

***Figure 2-3:*** *Table published in Kilvington et al. 2019 describing the phytosterol concentration reported in infant formula*

For example, fat in human milk makes up 50% of the total energy: to match that percentage, IFs need to be supplemented with external fat sources (Y. S. Chen, Aluwi, Saunders, Ganjyal, & Medina-Meza, 2019; Hageman, Danielsen, Nieuwenhuizen, Feitsma, & Dalsgaard, 2019). Other components naturally present in human milk (hormones, vitamins, and essential fatty acids) are also supplemented in IFs to achieve the correct infant dietary

requirements (Y. S. Chen et al., 2019). An interesting case is represented by long chain fatty acids (arachidonic acid, eicosapentanoic acid, and docosahexaenoic acid), which play a crucial role in cognitive and retinal development. IF with a fat composition that comes only from vegetable oil has higher levels of monounsaturated fatty acids and lower levels of medium chain fatty acids compared to human milk (Hageman et al., 2019). Recently, long chain polyunsaturated fatty acids, otherwise present in human milk, have been recently added to IFs as well (Y. S. Chen et al., 2019; Uauy & Dangour, 2009).

As it is stated above, there is significant evidence of the abundant presence of PS in IF, however, little is known about the effects of processing technologies on its oxidation (García-Llatas et al., 2008; Lagarda et al., 2006). The POP content in IF can be seen in Appendix B - Table 4. Because of the high lipid content in IF lipid due to fat supplementation with vegetable oils, oxidation is likely to occur. Few studies have investigated the formation of POPs within IF. Boatright and Crum (2016) tested three different infant formulations commonly found in the store and found that hydrogen peroxide, which is one of the ROS that can lead to lipid oxidation, was generated when preparing the formula according to the manufacturer's directions. Since the formation did not occur until after mixing with water, they concluded that the hydrogen peroxide was generated via a redox-cycling reaction from the initial ingredients inside the IF. This type of study emphasizes the need of perform more research focused on the importance of IF and the effects of its manufacturing in the formation of POPs.

New studies have started questioning the high PS content in IF, and hence, its potential oxidation process occurring during the infant formula manufacturing which generates POPs. Berger et al. (2004) recommended that children under five should not be

given phytosterols since they should have high cholesterol in their diets instead (Lemaire et al., 2018). Therefore, a question that arises is: Do we need to modify the IF recipe or the manufacturing process to dampen its lipid oxidative load?

## 2.7 PROCESSING TECHNOLOGY RELATED TO IF

Like most foods nowadays, IF undergoes rigorous processing prior to commercialization to guarantee the safety of the final product. IF is usually made by modifying cow's milk, with a variation in the whey-to-casein ratio (70/30). Whey proteins are predominant in breast milk (60% whey and 40% casein) and are believed to be more easily digested. Other substances that can be added are prebiotics (to aid digestion) and nucleotides. The former is normally found in breast milk and promotes brain and eye development (S. Damjanovic-Desic & Birlouez-Aragon, 2011; Traves, 2019). In general, the manufacturing process of IF involves a combination of different temperatures, pressures, and times, with a wide range varying between 60 and 200ºC, 0.8–20 MPa, and 30 s to 6 min, respectively. All these differences in the manufacturing procedures promotes the oxidation of the phytosterols present in the food matrix and produce potentially toxic compounds, such as POPs.

A general IF manufacturing process is shown in Figure 2-4. The main process steps are mixing, evaporation, and drying (Jiang & Guo, 2014). Temperature and pressure are the major parameters affecting the evaporation and drying processing steps. Evaporation, a critical process step for the removal of water, is preferred to spray drying since it requires less energy. More importantly, milk powder produced from evaporated milk has a longer shelf life and larger powder particles with a smaller amount of included air. Jiang and Guo (2014) explain that even though milk is commonly dried by roller drying or spray drying in a stream of hot air, spray drying is more commonly used for infant formula because roller

dried products have a lower solubility in water, are susceptible to irreversible component changes during drying, and because roller dried powder has a lower microbiological quality than spray dried powder.

Even though the overall manufacturing process of IF is basically the same, differences in temperatures and other processing parameters can still be observed. A patent filed in 1987 by Angel Gil and Luis Valverde, revealed the exact process used in the manufacturing of IF. The process starts mixing vegetable oil and aqueous products while heating to homogenize and emulsify the contents. Then the addition of nucleotides and other ingredients followed by pasteurized takes place at 95–100ºC. Next step uses vacuum to condense the mixture, with a slowly dry at a low temperature, then sterilized it twice at 121ºC or once at 151ºC for a few seconds (Gil, A., & Valverde, 1982). Another patent also states that infant formula is mixed and homogenized using high pressure and it is then spray dried at temperatures around 200ºC (Van Den Brenk, Van Dijke, Van Der Steen, Moonen, & Van Baalen, 2015). Moreover, Van Dijke, et al.'s patent describes the addition of a second emulsification step, pasteurization, or heat treatment of the aqueous phase from either 60 to 100ºC, or 70 to 90ºC, and more preferably to 85ºC, with a holding time of 1 s to 6 min, more preferably 10 s to 6 min, and even more preferably from 30 s to 6 min (Van Dijke, SCHRÖDER, USTUNEL, Reinhold HALSEMA, & Moonen, 2018). Moreover, they recommend that the lipid phase should be liquid at the temperature(s) used during the process. However, if the lipid phase is solid due to its composition it is preferably heated to above the melting temperature of at least one lipid, preferably vegetable lipid, contained in the lipid phase, specifically to a temperature above its melting point from 55 to 60ºC.

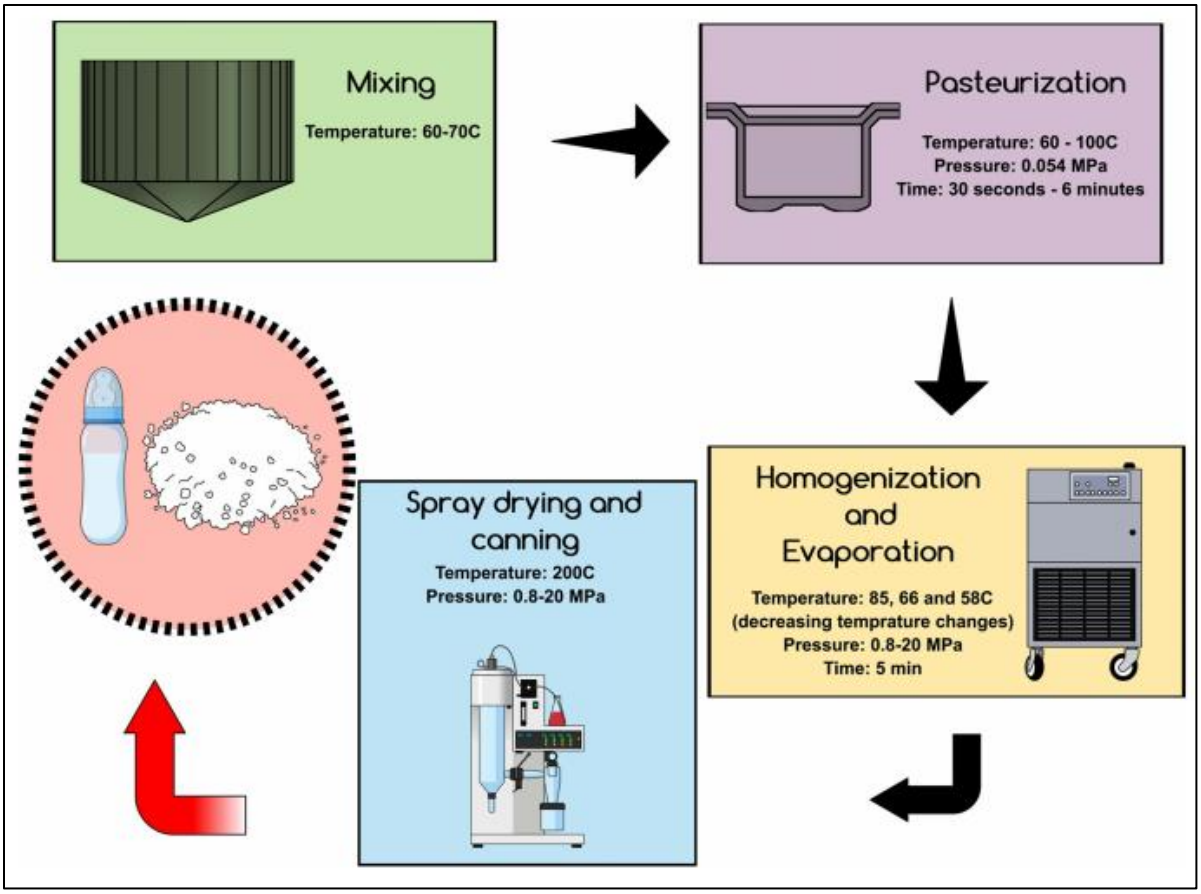


***Figure 2-4:*** *Infant formula milk powder manufacturing process (Kilvington et al. 2019)*

It is important to note that besides the variation of parameters involved in the manufacturing process previously mentioned, special attention should be given to other potential oxidation factors such as the IF packaging, handling, and preparation techniques at home that could promote the formation of POPs.

## 2.8 THE CHALLENGE OF FOOD PROCESSING: LIPID OXIDATION

Western diet contains levels of 150–400 mg/day of PS, being mainly β-sitosterols, campesterol, stigamasterol and traces of saturated phytostanols (Ryan et al., 2009). Therefore, POPs intake should be monitored to ensure low accumulation of these molecules in the human body, especially in infants, since like all unsaturated lipids, PS oxidation can

occur during the processing, preparation, and storage of IF (O'Callaghan et al., 2014). During a study of sterol oxidation in IF, it was found that the phytosterols were more oxidized than their animal counterpart, cholesterol (García-Llatas & Rodríguez-Estrada, 2011; González-Larena et al., 2011) pointing out the need of surveillance for these types of food. On the other hand, several studies have addressed the reduction of POPs with addition of antioxidants such as tocopherol, butylated hydroxytoluene (BHT), and plant ethanolic extracts (Rudzinska et al., 2005); however, no efforts have been dedicated to evaluating the different processing technologies and their effects in promoting PS oxidation in IF. It is imperative to build a strong scientific information base regarding not only PS consumption and its oxidized derivatives, but also their toxicity and exposure on a vulnerable population like infants.

Lipid oxidation is a major concern, but other nutritional components can also undergo reactions during food processing. The Maillard reaction is a well-known reaction that occurs in food during thermal processing. A reducing sugar and amino acid react together to form various Maillard reaction products (MRPs), depending on the conditions. The Maillard reactions leads to decreased nutritional value of proteins and some products may have adverse health effects. Milk and milk derivative products, like infant formula, are high in protein and sugar and so thermal processing of milk will lead to the formation of MRPs (Tamanna & Mahmood, 2015). Proteins can also undergo oxidation if exposed to heat, light, or metal. These oxidation reactions can lead to racemization of amino acids. This can lead to major changes in the protein's properties, activity, and structure which could also cause increased toxicity (Y. S. Chen et al., 2019). Lipid oxidation products can also react further with amino acids (Hematyar, Rustad, Sampels, & Kastrup Dalsgaard, 2019). The processing of infant formula can have drastic effects on the composition of the

ingredients inside. High heat leads to lipid and protein oxidation, as well as the Maillard reaction. These reactions can lead to the formation of a wide range of components that can further react and may have drastic effects on infants.

## 2.9 CONCLUSIONS

PS are plant sterols present in vegetable food sources. Their oxidation is promoted by temperature, storage, and processing, generating oxidized derivatives know as POPs. IF is given to infants at a very early age, especially those who have special dietary restriction or medical conditions and cannot fulfill their nutritional needs from breast milk. These young children rely on the contents of infant formula to obtain the nutrition needed for a healthy and complete physical development and growth. However, the scarce information related with POPs, the mechanisms these compounds undergo, and the potential adverse health effect on infants that could result from their intake through infant formula consumption is still unclear and should be of great concern to the scientific community. What is the outcome when the only meal these young children have is filled with oxidized sterols that could affect them for the rest of their life? Even though there are not enough studies of POPs formation due to food processing, there is no doubt that the processing of IF leads to the formation of oxidized sterol products. Therefore, high amounts of phytosterols in infant formula could result in high concentrations of POPs. The effects of infant formula processing in the POPs formation mechanism and their connection with infant's health are still widely unknown. However, it is well known that they can have consequences on human health based on studies performed in different food matrices. A deeper analysis of the processing and treatment of infant formula is crucial to obtain a better understanding of these

compounds' formation pathways and their biological mechanism in infants, which are a critical and vulnerable population.

# CHAPTER 3 : EVALUATION OF THE NUTRITIONAL QUALITY OF ULTRA-PROCESSED FOODS (READY TO EAT + FAST FOOD): FATTY ACID COMPOSITION

The content of this chapter has been submitted for revision to the Food and Chemical Toxicology Journal. DOI: 10.1101/2021.04.16.21255610

## 3.1 INTRODUCTION

Dietary lipids provide up to 42% of the calories ingested in the Western diet, whereas nutritional recommendations of fat for adults are 20–35% (Niot, Poirier, Tran, & Besnard, 2009). In the Western diet, ~95% of dietary lipids are comprised of triacylglycerols, (mainly long-chain fatty acids (LCFA)), and the remaining 5% includes phospholipids (4.5%) and cholesteryl esters (0.5%). Adherence to a "Westernized" dietary pattern is characterized by the consumption of high amounts of processed meats (F. B. Hu, 2002; Schulze & Hu, 2002). Nearly 60% of the calories consumed by the average North American individual are obtained from the intake of ultra-processed foods (UPFs) (Martínez Steele et al., 2016; Urban et al., 2016). Fast foods (FF) (including small, large, and non-chain restaurant) and Ready to Eat (RTE) meals constitute the people's top choices. A healthy human with an average of 70 kg of body weight, stores around 141,000 kcal as fat as compared to 24,000 kcal as protein and 1,000 kcal as carbohydrate (Wang, Liu, Portincasa, & Wang, 2013). Furthermore, dietary fat is the most calorically dense macronutrient, supplying 9 kcal/g, about double of what is contributed by either protein or carbohydrate at 4 kcal/g. The increase of consumption of dietary fat from UPFs related to a qualitative imbalance (excess of saturated fatty acids and cholesterol) has been associated with the increased risk in the development

of several chronic diseases such as obesity, diabetes, cancer, and cardiovascular disease (Zinocker & Lindseth, 2018).

Food processing does not represent an issue for human nutrition, neither does it inherently cause negative health outcomes. However, the over-processing of food can lead to the accumulation of harmful substances formed because of thermal treatments, light exposure, storage, and aging food, the denaturalization of proteins, vitamins, and bioactive components, compromising the overall nutritional quality.

Processed foods (PFs) and UPFs are food items considered as inexpensive, *highly processed,* rich in calories, but low in some essential micronutrients such as mineral and vitamins. Studies have shown that UPFs comprise 57.9% of the energy intake (Martínez Steele et al., 2016). UPFs are mostly consumed away from home and have been proposed as a major contributor to the energy intake rise (Urban et al., 2016). Another big group of meals highly consumed within the Western diet are the RTE meals. RTE meals are defined as any food that is either normally eaten in its raw state or has been somehow processed, for which it is reasonably foreseeable that the food will be eaten without further processing that would significantly minimize biological hazards (FDA, 2020). Usually, RTE foods are packed processed products for sale which require minimum preparation at home.

Most foods from the Western diet, including FFs and RTE meals, belong to the PFs or UPFs category according to the NOVA (*not an acronym*) classification system (C. A. Monteiro, Cannon, Lawrence, Costa Louzada, & Pereira Machado, 2019). These terms differ in the number of processing techniques applied during the production of the food item. NOVA is recognized by the Food and Agriculture Organization (FAO) and Pan-American Health Organization (PAHO) as a valid tool for nutrition and public health research and "categorizes

foods according to the extent and purpose of food processing, rather than in terms of nutrients." (Juul et al., 2018; Martínez Steele et al., 2016; Moubarac et al., 2017). Consumers are continuously exposed to "new" PFs and UPFs. From a nutritional perspective, food processing emerges as a critical topic for public health linking nutritional quality and food safety.

The aim of this chapter is to report the fat content and fatty acids profile of a selection of UPFs, to establish a database useful not only for nutritional and clinical interventions, but also to improve food chemical safety and nutritional food quality. UPFs were analyzed based on fat sources, type of food and correlations between the different components among the studied food categories to establish a possible trend related to the relationship with fat source, sugars, salt, and calorie content.

## 3.2 MATERIALS AND METHODS

### 3.2.1 Materials, Chemicals, and Reagents

Methanol was from Sigma-Aldrich (St. Louis, MO). Chloroform was obtained from Omni Solv (Burlington, MA), hexane was purchased from VWR BDH Chemicals (Batavia, IL), 1-butanol and potassium chloride (KCl) from J. T. Baker (Allentown, PA) and diethyl ether was purchased from Fisher Chemical (Pittsburgh, PA). Sodium sulfate anhydrous ($Na_2SO_4$) and sodium chloride (NaCl) were also purchased from VWR BDH Chemicals. Supelco 37 FAME standard mixture was purchased from Sigma Aldrich (St Louis, MO).

### 3.2.2 Sample Collection

Composite foods and food products of different categories of UPFs were collected from retail stores, supermarkets, food chains, restaurants, and takeaway in Lansing area (Michigan, USA) between February 2018 and October 2019. Different brands and different

retailers for the same type of food were acquired to achieve a representative sample. A complete list of the food meals, their respective test code, and group is provided in Appendix C. Meals were classified in two main groups: Fast Foods (FFs) and Ready to Eat (RTE), where 23 FF meals were purchased from the eight most popular franchisees in the state of Michigan covering more than 75% of the national market (Dunford et al., 2017; Powell et al., 2019; Rwithley, 2019; Tran et al., 2019). In addition, food items and meals were grouped in subcategories according to the fat source as follow: eggs and egg's derivatives (E), dairy products (D), meat and poultry (MP), seafood (S), baby food (BF). Additional food items that did not fit in any of the previous categories, such as potato-products (potato crisps with and without added flavors, French fries from restaurants and takeaway, frozen potatoes pre-fried and fried, and homemade French fries), pasta, salad dressings, and popcorn (sweet or salty) were grouped as other products (O). Once the UPF arrived at the laboratory, an excel form was filled out with the following information: (1) item's name; (2) price; (3) place, date, and time of collection; (4) type of food (RTE or FF); (5) nutritional declaration (energy, fat, saturated fatty acids, carbohydrates, sugars, fiber, protein and salt); (6) portion size; (7) list of ingredients; (8) expiring date; and (9) other relevant information.

### 3.2.3 Sample Preparation

FF meals were purchased from each individual franchise and brought to the laboratory for immediate analysis. RTE meals were purchased from different local supermarket stores, and immediately brought to the laboratory. Storage conditions were followed according to the label instructions (fresh foods were kept in a fridge at 4 °C and frozen meals were kept at -20 °C or the temperature indicated in the label). All meals were analyzed before the expiration date. When further cooking procedures were required, items were prepared in accordance with manufacturer's instructions. Kitchen equipment available

at the Michigan State University's Food Science Laboratory was supplied and used for this study. All the samples were homogenized using an Ultra-Turrax® (Tekmar TP 18/10S1 Cincinnati, OH) at least for 3 min at 5000 rpm, split and stored accordingly, depending on the food matrix.

### 3.2.4 Lipid Extraction

Lipid fraction was extracted according to the Folch cold extraction method (Folch, Lees, & Sloane Stanley, 1957) with some modifications depending the food matrix. Thirty grams of sample were minced and placed in a 500 mL glass bottle with screwcap where 200 mL of a chloroform:methanol solution (1:1, v/v) was added. Sample was mixed for 15 min at 300 rpm. Homogenization was performed using an Ultra-Turrax for 3 minutes. The bottle was kept in an oven at 60°C for 20 min before adding 100 mL chloroform. After 2 min of vortex mixing the sample, the content of the bottle was filtered. The filtrate was mixed thoroughly with a 100 mL of 1 M KCl solution, and left overnight at 4 °C. Then, the lower phase containing lipids was collected and dried at 60°C with a multi-vacuum solvent evaporator (Organomation S-EVAP-RB, Berlin MA) at 25 inches of Hg. Total fat content was determined gravimetrically.

### 3.2.5 Fatty Acid Methyl Esters (FAME)

Fatty acid methyl esters (FAME) were prepared according to the transesterification described in Chen's procedure (Y. S. Chen et al., 2019). One µL of the methylated sample was injected into a gas chromatography (GC 2010 Shimadzu, Kyoto Japan) equipped with a DB-WAX capillary column (30 m × 0.32 mm i.d. × 0.25 µm). The oven temperature gradient was set as follows: 120 °C to 200°C with a rate of 3°C/min, then from 200 °C to 240°C with a rate of 2 °C/min and held for 2 min. Injector and detector were both set at 250°C. $H_2$

was used as carrier gas at 1mL/min and split ratio 5.0. Data acquisition was done by Lab solutions software (Shimadzu, Kyoto, Japan) and peaks areas were identified by comparing their retention times to the pure standards. Standard curves of FAME 37 mixture were built with different concentrations from 65 to 500 ug/mL. Fatty acid contents were reported as the weight percentage of total fatty acid detected (% w/w) per g of fat.

Results on fatty acids contents were obtained using the sums: Σ saturated fatty acids (Σ SFA = C8:0 + C10:0 + C11:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 + C22:0 + C23:0 + C24:0); Σ monounsaturated fatty acids (Σ MUFA = C14:1, cis-9 + C15:1, cis-10 + C16:1, cis-9 + C17:1, cis-10 + C18:1, cis- 9 + C20:1, cis-11 + C22:1, cis-13 + C24:1, cis-15); Σ polyunsaturated fatty acids (Σ PUFA = C18:2, cis-9,12 + C18:3, cis-6,9,12 + C18:3, cis-9,12,15 + C20:2, cis-11,14 + C20:3, cis-8,11,14 + C20:3, cis-11,14,17 + C20:4, cis-5,8,11,14 + C22:2, cis-13,16 + C20:5, cis-5,8,11,14,17 + C22:6, cis-4,7,10,13,16,19). and Σ trans fatty acids (Σ TFA= C18:1, trans-9 + C18:2, trans-9,12).

### 3.2.6 Statistical Analysis

Descriptive statistics were calculated overall and by category. Both mean, and confidence interval (95%) were reported. Since the data did not follow a normal distribution, when comparing RTE vs FF items, a Matt-Whitney *U*-test was performed, at $p < 0.05$ significance level. Statistical differences between food categories were evaluated by means of the non-parametric Kruskal-Wallis ANOVA by Ranks test, followed by post-hoc comparisons of mean ranks of all pairs of groups. Before statistical assessment, FAME percentages were arcsine square root transformed (Olson, 1976). All the statistical analysis were computed using SPSS v.26 (IBM).

## 3.3 RESULTS AND DISCUSSION

### 3.3.1 Total Fat, Sugar and Sodium in UPFs

The overall total fat ranges from 0.60 to 87.62 g/100 g of product with the dairy category (n=11) being the group with the highest fat content followed by the eggs and egg's derivatives category (n=2) with up to 77 g/100 g of product (Table 3-1).

Figure 3-1 (A-D) shows a boxplot distribution of fat and FAME groups within each category. UPFs meals and food products were grouped based on their fat source as describe above. Some food products like edible fats (olive, avocado, canola, etc.) were not included since they are considered foods themselves with no additional components. Due to the large number of UPFs in this study, total fat will be primarily discussed for each food category, detailing it for individual meals only when a distinct result is statistically significant. FF and RTE meals are known to be served in large portions, containing high levels of saturated fat, and added sugars (Harris et al., 2013; Rosenheck, 2008). Most of these meals are prepared with several ingredients such as oils, eggs, high fat meats - all of them containing high fat content themselves - resulting in a meal with both high fat and high caloric content.

### 3.3.2 Fatty Acid Profile by Food Category

Fatty acid composition of RTE and FF meals is shown in Appendix D. Thirty-five fatty acids were identified and quantified. Short chain fatty acids (SC-FA) were not reported since C4 and C6 co-eluded with the solvent. Also, dihomo-γ-linolenic acid (C20:3n-3, DGLA) and eicosatrienoic acid (C20:3n-6) were not chromatographically resolved for some samples, therefore their area values were merged and reported as C20:3n-3 + C20:3n-6. Tables 3-2 to 3-4 show the percentage of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), respectively.

***Table 3-1:*** *Food ID used in this study and unpublished results of total fat, sugar, sodium, and calories per serving found in the different UPFs.*

| Category | Food ID | Total Fat (g/100 g sample) | Total Fat Nutritional label ** (g/100 g sample) | Serving Size | Total Fat (g) per serving size | Sugar (g) per serving size | Sodium (mg) per serving | Calories per serving |
|---|---|---|---|---|---|---|---|---|
| **Ready to Eat** | | | | | | | | |
| Dairy | D1 - RTE | 17.67 ± 2.45 | 18.40 | 19.00 g | 3.50 | 1.00 | 280 | 60 |
| | D2 - RTE | 26.92 ± 8.47 | 33.00 | 21.00 g | 6.93 | 0 | 180 | 110 |
| | D3 - RTE | 49.71 ± 2.56 | 50.00 | 1 tbsp (14.15 g) | 7.08 | NR | 100 | 60 |
| | D4 - RTE | 87.62 ± 1.91 | 78.57 | 14.00 g | 11.00 | 0 | 90 | 100 |
| | D5 - RTE | 10.27 ± 0.38 | 10.00 | 30.00 mL (28.3 g) | 2.83 | 1 | 25 | 40 |
| | D6 - RTE | 24.16 ± 6.98 | 28.00 | 28.00 g | 7.84 | 1 | 240 | 60 |
| | D7 - RTE | 42.82 ± 1.68 | 35.00 | 28.00 g | 9.80 | 2 | 95 | 90 |
| | D8 - RTE | 12.78 ± 3.04 | 23.40 | 0.50 cup (65.00 g) | 15.21 | 14 | 40 | 130 |
| | D9 - RTE | 1.60 ± 0.31 | 3.03 | 1 container (99.00g) | 3.00 | 20 | 90 | 150 |
| | D10 - RTE | 2.14 ± 0.54 | 0.96 | 414.03 g | 0.60 | 37 | 230 | 250 |
| | D11 - RTE | 12.56 ± 0.38 | 0.69 | 1 scoop (9.00 g) | 0.062 | NR | 0.32 | 44 |
| Meat & Poultry | MP1 - RTE | 23.45 ± 5.00 | 20.00 | 38.00 g | 7.60 | < 1 | 260 | 90 |
| | MP2 - RTE | 17.84 ± 2.65 | 26.00 | 32.00 g | 8.30 | 0 | 260 | 50,000 |
| | MP3 - RTE | 0.60 ± 0.071 | 0.65 | 240.00 mL (226.40 g) | 1.50 | 3 | 870 | 170 |
| | MP4 - RTE | 3.64 ± 0.018 | 3.30 | 1 can (236.59 g) | 7.80 | 5 | 870 | 250 |
| | MP5 - RTE | 3.53 ± 0.55 | 5.65 | 11.00 oz (227.00 g) | 12.80 | 5 | 500 | 270 |
| | MP6 - RTE | 1.37 ± 0.31 | 1.76 | 0.5 cup (113.40 g) | 2.00 | 0 | 640 | 60 |
| | MP7 - RTE | 3.61 ± 0.013 | 0.61 | 0.5 cup (240.00 g) | 1.50 | 1 | 600 | 70 |
| | MP8 - RTE | 2.91 ± 0.53 | 2.87 | 1 cup (249.00 g) | 7.15 | 6 | 800 | 200 |
| | MP9 - RTE | 4.61 ± 0.097 | 4.28 | 1 cup (257.00 g) | 11.00 | 8 | 800 | 260 |
| Seafood | S1 - RTE | 3.62 ± 0.25 | 3.38 | 18.80 oz (532.97 g) | 18.01 | 1 | 890 | 180 |
| Egg's Derivatives | E1 - RTE | 77.68 ± 1.95 | 75.00 | 1 Tbsp (13 g) | 9.75 | NR | 90 | 90 |
| | E2 - RTE | 14.64 ± 1.02 | 13.91 | ½ cup (115.00 g) | 16.00 | 10 | 330 | 380 |
| Baby Food | BF1 - RTE | 1.80 ± 0.15 | 1.41 | 71.00 g | 1.00 | 0 | 35 | 50 |
| | BF2 - RTE | 7.28 ± 0.19 | 4.40 | 4.00 oz (113.40 g) | 4.99 | NS | 40 | 90 |

*Table 3-1 (cont'd)*

| Category | Food ID | Total Fat (g/100 g sample) | Total Fat Nutritional label ** (g/100 g sample) | Serving Size | Total Fat (g) per serving size | Sugar (g) per serving size | Sodium (mg) per serving | Calories per serving |
|---|---|---|---|---|---|---|---|---|
| **Ready to Eat** | | | | | | | | |
| Baby Food | BF3 - RTE | 2.99 ± 0.06 | 4.40 | 4.00 oz (113.40 g) | 4.99 | 3 | 30 | 70 |
| | BF4 - RTE | 3.25 ± 0.23 | 4.40 | 4.00 oz (113.40 g) | 4.99 | 3 | 40 | 70 |
| | BF5 - RTE | 3.23 ± 0.14 | 4.40 | 4.00 oz (113.40 g) | 4.99 | 3 | 45 | 80 |
| | BF6 - RTE | 2.12 ± 0.60 | 1.56 | 128.00 g | 2.00 | 5 | 260 | 120 |
| | BF7 - RTE | 3.09 ± 0.16 | 4.40 | 4.00 oz (113.40 g) | 4.99 | 5 | 20 | 80 |
| | BF8 - RTE | 6.00 ± 0.14 | 4.90 | 71.00 g | 3.48 | 0 | 20 | 50 |
| | BF9 - RTE | 2.79 ± 0.44 | 4.40 | 4.00 oz (113.40 g) | 4.99 | 11 | 50 | 100 |
| | BF10 - RTE | 4.63 ± 0.48 | 4.40 | 4.00 oz (113.40 g) | 4.99 | 4 | 40 | 80 |
| | BF11 - RTE | 3.89 ± 0.23 | 4.40 | 4.00 oz (113.40 g) | 4.99 | 4 | 80 | 120 |
| | BF12 - RTE | 2.90 ± 0.38 | 4.40 | 4.00 oz (113.40 g) | 4.99 | 6 | 75 | 90 |
| | BF13 - RTE | 1.39 ± 0.25 | 2.35 | 85.00 g | 2.00 | 1 | 170 | 80 |
| Other | 01 - RTE | 19.97 ± 1.85 | 26.60 | 2 tbsp (30.00 g) | 7.98 | 0 | 290 | 140 |
| | 02 - RTE | 45.11 ± 6.32 | 50.00 | 2 tbsp (30.00g) | 15.00 | 1 | 230 | 140 |
| | 03 - RTE | 8.70 ± 5.00 | 5.00 | 1 package (58.00 g) | 2.90 | 6 | 570 | 250 |
| | 04 - RTE | 3.65 ± 1.51 | 5.00 | 2.5 oz (70.90 g) | 3.55 | 4 | 500 | 220 |
| **Fast Food** | | | | | | | | |
| Meat & Poultry | MP10 - FF | 33.13 ± 5.12 | 10.00 | 95.00 g | 9.50 | NR | 510 | 250 |
| | M11 - FF | 19.52 ± 1.46 | 20.00 | 4 pieces (64.00 g) | 12.80 | NR | 330 | 170 |
| | MP12 - FF | 23.59 ± 3.40 | 14.00 | 119.00 g | 16.66 | NR | 720 | 300 |
| | MP13 - FF | 9.83 ± 0.45 | 11.40 | 1 taco (102.00 g) | 11.63 | 1 | 306 | 269 |
| | MP14 - FF | 11.06 ± 2.09 | 7.60 | 1 quesadilla (170.00 g) | 12.92 | 0 | 310 | 180 |
| | MP15 - FF | 9.95 ± 0.67 | 9.90 | 1 burrito (140.00 g) | 13.86 | 1 | 1650 | 640 |
| | MP16 - FF | 12.48 ± 0.30 | 12.21 | 1 piece (75.00 g) | 9.16 | 0 | 430 | 130 |

*Table 3-1 (cont'd)*

| Category | Food ID | Total Fat (g/100 g sample) | Total Fat Nutritional label ** (g/100 g sample) | Serving Size | Total Fat (g) per serving size | Sugar (g) per serving size | Sodium (mg) per serving | Calories per serving |
|---|---|---|---|---|---|---|---|---|
| Fast Food | | | | | | | | |
| Meat & Poultry | MP17 - FF | 19.39 ± 2.55 | 18.60 | 1 piece (60.00 g) | 11.16 | 0 | 380 | 130 |
| | MP18 - FF | 6.26 ± 1.21 | 4.58 | 5.40 oz (153.09 g) | 7.01 | 7.00 | 520 | 150 |
| | MP19 - FF | 8.19 ± 0.78 | 10.80 | 5.70 oz (161.69 g) | 17.46 | 19.00 | 820 | 490 |
| | MP20 - FF | 14.23 ± 2.82 | 10.50 | 1 sandwich (187.00 g) | 19.64 | 9.00 | 680 | 320 |
| | MP21 - FF | 7.12 ± 1.18 | 6.51 | 1 sandwich (71.28 g) | 4.64 | 3 | 2370 | 810 |
| | MP22 - FF | 10.74 ± 4.18 | 8.79 | 1 sandwich (94.61 g) | 8.32 | 2 | 1940 | 850 |
| | MP23 – FF*** | 12.59 ± 1.35 | 11.50 | 1 slice (123.00 g) | 14.15 | 3 | 950 | 370 |
| | MP24 - FF | 10.96 ± 2.70 | 11.50 | 1 slice (79.00 g) | 9.09 | 2.00 | 740 | 380 |
| Seafood | S2 - FF | 22.82 ± 1.07 | 14.08 | 1 sandwich (131.00 g) | 18.44 | NR | 580 | 380 |
| | S3 - FF | 21.70 ± 0.15 | 13.10 | 5.00 oz (141.75 g) | 18.57 | 14.00 | 440 | 360 |
| Other | 05 - FF | 13.92 ± 1.58 | 20.00 | 1 medium serving (117.00 g) | 23.40 | NR | 260 | 320 |
| | 06 - FF | 16.27 ± 2.86 | 16.00 | 1 biscuit (76.00 g) | 12.16 | NR | 810 | 260 |
| | 07 - FF | 6.29 ± 1.34 | 6.00 | 3 hotcakes (149.00 g) | 8.94 | NR | 550 | 580 |
| | 08 - FF | 18.16 ± 0.75 | 17.30 | 1 biscuit (49.00 g) | 8.48 | 1 | 520 | 180 |
| | 09 - FF | 15.96 ± 0.21 | 15.00 | 1 order (34.99 g) | 5.25 | 0 | 1100 | 320 |
| | 010 - FF | 1.46 ± 0.52 | 3.11 | 1 order (16.85 g) | 0.52 | 0 | 520 | 130 |

**Information found in the USDA Food Database. www.fdc.nal./usda.gov/fdc-app.html#/
***Information found in Menuwithprice.com. Not available in neither USDA Food Database nor Marco's Pizza website.
NR = not reported
NS = not a significant source of the nutrient

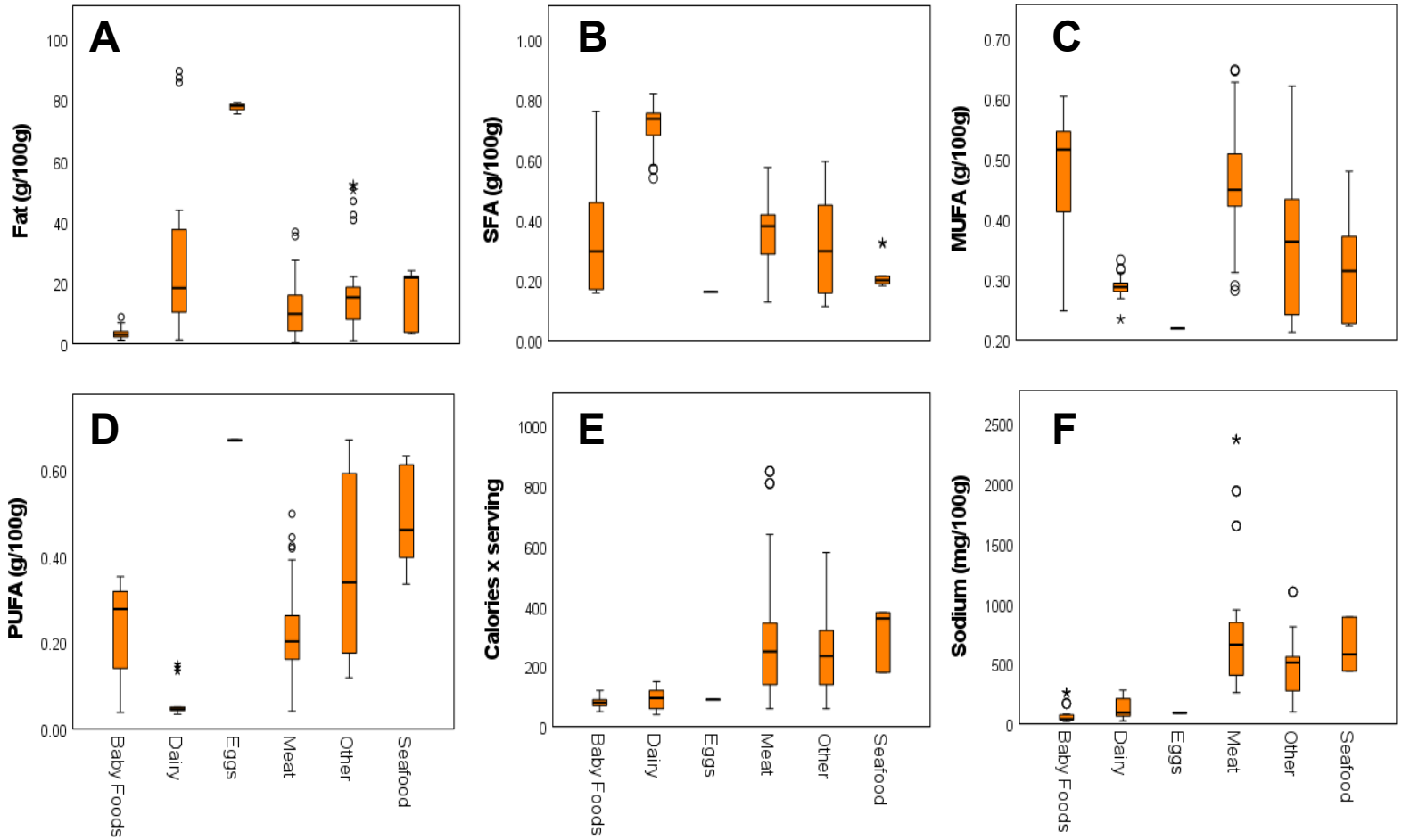


***Figure 3-1:*** *Unpublished results of (A) fat content vs. food categories, (B) SFA vs. food categories, (C) MUFA vs. food categories, (D) PUFA vs. food categories, (E) calories per servings vs. food categories, and (F) sodium vs. food categories*

Figure 3-1 (B-D) depicts the overall distribution of FAME categories (SFA, MUFA, PUFA) according to the food source. No statistically significant differences were found between FF and RTE groups.

The meat and poultry category (n=24) which includes beef, chicken, pork, turkey, and combinations of these meats, had the third largest amount of total fat (values ranging from 0.60 to 33.13 g/100 g of product). The majority of UPFs showed a higher fat content compared to the values reported either in their nutritional label or website. Higher fat contents were observed in several dairy products containing butter as part of its use in their preparation process. Butter is the only processed food included in this study due to the great use in the manufacturing of several UPFs.

### 3.3.3 Saturated Fatty Acids (SFA)

Saturated fatty acid was the overall second major content of fatty acids between both FF and RTE meals with 35.57%, just behind the monounsaturated fatty acids (37.82%). The total SFA content was significantly different between RTE and FF with 40.88 and 29.88 g/100g of product, respectively (Table 3-2). C16:00 was the predominant SFA in both RTE and FF meals with a mean of 21.74 g/100 g, followed by C18:00 with 7.61 g/100g. Regarding the length of the saturated chain in the fatty acid, long chain fatty acid (LC-SFA) accounted for more than 90% of the total SFA Content in comparison with 5.3% of the medium chain saturated fatty acid (MC-SFA). Dietary triacylglycerols are derived principally from two sources: animal fats and vegetable oils. Animal fats contain a high proportion of SFA as for butter, which SFA content mostly consists in 4 SFAs (C12:00, C14:00, C16:00, and C18:00) (Bobe, Hammond, Freeman, Lindberg, & Beitz, 2003; Ledoux et al., 2005; Lopes, Cañedo, Oliveira, & Alcantara, 2018). On the other hand, vegetable oils have a higher

proportion of unsaturated fatty acids. Since LCFA are hydrophobic nutrients, their intestinal absorption is a complex process. Fatty-acid chain length and unsaturation number influence fat absorption. In human diet, approximately 95% of dietary lipids are triacylglycerols (TAG), mainly composed of long-chain fatty acids (LCFA, number of carbons >16). Medium-chain fatty acids (MCFA) are better absorbed than LCFA because they can be solubilized in the aqueous phase of the intestinal contents, absorbed bound to albumin, and transported to the liver by the portal vein (Wang et al., 2013). Further, absorption in the stomach occurs after the hydrolysis of medium-chain triglycerides (MCT) by gastric lipase, enhancing their solubilization in the intestine, where they are absorbed bound to albumin and transported to the liver via the portal vein (Niot et al., 2009). LCFA exert basic functions in the cell as membrane components, metabolic fuel, precursors of lipid mediators, regulators of ion channels and modulators of gene expression (Linder & Deschenes, 2007). They participate in several post-translational protein modifications (e.g., palmitoylation) affecting their cellular functions. Moreover, dietary lipid fecal loss remains below 5% (w/w), still with a high fat intake in healthy individuals (Ramírez, Amate, & Gil, 2001). Hence, lipid uptake is not a rate-limiting step for the intestinal fat absorption, and more attention should be paid to the meals size portion and fat source to maintain a healthy diet.

Interestingly, albeit not surprising considering the lipid needs for infants, infant formula has the overall highest SFA content from all UPFs with 56%. SFA content in infant formulas containing dairy as their major source of fat is significantly higher in comparison with those having plant oils as their main source (C. Sun, Wei, Su, Zou, & Wang, 2018).

*Table 3-2: Unpublished results of the saturated fatty acid composition in RTE and FF meals*

| SFA | Total | Ready-To-Eat | Fast-Food | Mann-Whitney U-test |
|---|---|---|---|---|
| C8:0 | 1.18 (0.08-1.59) | 1.43 (0.08-2.03) | 0.64 (0.49-0.80) | |
| C10:0 | 1.74 (1.49-2.00) | 2.01 (0.17-2.36) | 1.22 (1.03-1.41) | * |
| C11:0 | 0.06 (0.05-0.08) | 0.07 (0.06-0.09) | 0.04 (0.02-0.05) | *** |
| C12:0 | 2.05 (1.63-2.46) | 2.04 (1.57-2.51) | 2.06 (1.22-2.89) | |
| C14:0 | 3.54 (2.99-4.09) | 4.22 (3.43-5.02) | 2.36 (1.81-2.91) | ** |
| C15:0 | 0.42 (0.36-0.49) | 0.51 (0.42-0.06) | 0.28 (0.21-0.34) | * |
| C16:0 | 21.74 (20.52-22.96) | 23.96 (22.34-25.57) | 18.09 (16.57-19.60) | **** |
| C17:0 | 0.35 (0.31-0.39) | 0.39 (0.34-0.44) | 0.29 (0.22-0.35) | ** |
| C18:0 | 7.61 (7.10-8.12) | 8.41 (7.69-9.13) | 6.29 (5.76-6.82) | *** |
| C20:0 | 0.29 (0.26-0.32) | 0.24 (0.21-0.28) | 0.35 (0.31-0.40) | *** |
| C21:0 | 0.30 (0.24-0.35) | 0.31 (0.22-0.40) | 0.27 (0.23-0.32) | |
| C22:0 | 0.12 (0.08 -0.15) | 0.12 (0.07-0.17) | 0.11 (0.06-0.17) | |
| C23:0 | 0.05 (0.03-0.08) | 0.03 (0.02-0.04) | 0.07 (0.03-0.10) | *** |
| C24:0 | 0.22 (0.13-0.32) | 0.21 (0.06-0.35) | 0.24 (0.10-0.37) | *** |
| Σ SFA | 35.57 (33.01-38.13) | 40.88 (37.48-44.29) | 29.88 (27.17-32.60) | **** |
| Σ MC-SFA | 3.66 (2.98-4.34) | 3.94 (3.05-4.82) | 3.08 (2.06-4.10) | |
| Σ LC-SFA | 33.66 (31.59-35.72) | 37.31 (34.48-40.15) | 27.61 (25.36-29.86) | **** |

ΣSFA: Sum of saturated fatty acid, ΣMC-FA: sum of medium chain saturated fatty acid, ΣLC-FA long chain saturated fatty acid.

Even though the majority of SFA in both RTE and FF meals were straight chain even-numbered homologues (i.e., C12:00, C14:00, C16:00, etc.), C15:00, C17:00, and C23:00 were also detected in UPFs, being C15:00 the only statistically significant. Dairy foods, comprised of RTE items, contain the highest amount of C15:00 with 1.02 g/100g of product followed by the baby foods category. All type of cheeses (American, Cheddar, and Swiss) showed a high SFA content which agrees with literature (Manuelian, Currò, Penasa, Cassandro, & De Marchi, 2017); except for the cream cheese, which literature values were

not available to confirm their high SFA content. Mac & Cheese (boiled, prepared) showed a higher fat content than the Mac & Cheese prepared by microwave heating. These results demonstrate that cooking techniques and preparation are critical parameters that should be considered when evaluating nutritional quality of foods. Total fat content for butter and ice cream agreed with previous studies (Nielsen, 1996a; Tavella et al., 2000) and the USDA food database (USDA, 2018).

The UPFs classified as "Others" (French fries, biscuit, hotcakes, mashed potato) contained 30.7% SFA, which was confirmed with their reference values in the USDA database (Huang et al., 2017; USDA, 2019b, 2019c). Palmitic acid (C16:0), lauric acid (C12:0), stearic acid (C18:0), and myristic acid (C14:0) were the four leading FA between the "Other" category.

RTE and FF meals are the UPFs with a major contribution of fat accumulation in the human body in the Western diet (Mohiuddin, 2019), which is directly associated to different chronic diseases such as obesity, diabetes, hypertension, atherosclerosis, among others (Shori, Albaik, & Bokhari, 2017). Therefore, the quantification of total fat in RTE and FF meals is important to evaluate the actual dietary fat intake of adults and children who consume large amount of UPFs.

### 3.3.4 Monounsaturated Fatty Acids (MUFA)

MUFA was the group with the highest content among all UPFs. FF values ranged from 40 to 45 (g/100 product) (Table 3-3) while RTE values range from 34 to 38 (g/100 product). It is worth mentioning that RTE meals contain higher percentages of SFA (40.88 g/100 of product) than the overall percentage of MUFA among UPFs. Conversely, the FF group contains the highest percentage of MUFA than the overall amount of both SFA and MUFA in

all UPFs. Furthermore, the oleic acid (C18:1, cis-9, OA) was the dominant fatty acid in all UPFs with more than 90% of the total MUFA. OA not only provides energy but also reduces the melting point of triacylglicerides (Ramírez, Amate, & Gil, 2001). Baby foods and meat & poultry were the categories with the higher content of OA, followed by seafood.

***Table 3-3:*** *Unpublished results of monounsaturated and polyunsaturated fatty acid composition in RTE and FF meals*

| *MUFA* | Total | Ready-To-Eat | Fast-Food | Mann-Whitney U-test |
|---|---|---|---|---|
| *C14:1* | 0.55 (0.48-s0.62) | 0.59 (0.52-0.67) | 0.46 (0.32-0.60) | *** |
| *C15:1* | 0.18 (0.12-0.25) | 0.18 (0.12-0.25) | ND | - |
| *C16:1* | 1.76 (1.56-1.96) | 1.83 (1.57-2.09) | 1.63 (1.31-1.95) | |
| *C17:1* | 0.26 (0.22-0.31) | 0.26 (0.20-0.31) | 0.28 (0.18-0.38) | |
| *tC18:1* | 1.23 (0.77-1.70) | 2.14 (0.53-3.75) | 0.93 (0.59-1.27) | |
| *C18:1 n-9* | 36.09 (34.65-37.53) | 33.64 (31.88-35.39) | 40.15 (37.92-42.38) | **** |
| *C20:1* | 0.40 (0.36-0.44) | 0.33 (0.28-0.39) | 0.49 (0.42-0.55) | **** |
| *C22:1* | 0.28 (0.20-0.36) | 0.19 (0.15-0.24) | 0.34 (0.21-0.47) | *** |
| *C24:1* | 0.19 (0.09-0.28) | 0.14 (0.05-0.23) | 0.27 (0.02-0.57) | *** |
| *ΣMUFA* | 37.82 (36.01-39.63) | 36.57 (34.64-38.50) | 43.17 (40.73-45.61) | **** |

| PUFA | Total | Ready-To-Eat | Fast-Food | Mann-Whitney U-test |
|---|---|---|---|---|
| *tC18:2* | 0.35 (0.30-0.40) | 0.39 (0.31-0.46) | 0.31 (0.23-0.39) | |
| *C18:2 n-6* | 20.88 (18.81-22.95) | 19.59 (16.88-22.29) | 23.02 (19.82-26.22) | * |
| *C18:3n-6* | 0.10- (0.08-0.12) | 0.11 (0.08-0.14) | 0.09 (0.07-0.10) | |
| *C18:3n-3* | 2.70 (2.37-3.03) | 2.7 (2.21-3.14) | 2.75 (2.31-3.19) | |
| *C20:3n3 + C20:3n6* | 0.12 (0.10-0.13) | 0.12 (0.10-0.15) | 0.11 (0.10-0.13) | |
| *C20:4n-6* | 0.11 (0.05-0.17) | ND | 0.11 (0.05-0.17) | |
| *C20:5n-3* | 0.27 (0.16-0.39) | 0.21 (0.12-0.30) | 0.32 (0.12-0.51) | *** |
| *C22:2 n-6* | 0.06 (0.00-0.13) | 0.02 (0.01-0.04) | 0.13 (-0.13-0.40) | **** |
| *C22:6 n-3* | 0.05 (0.04-0.07) | 0.05 (0.03-0.07) | 0.06 (0.04-0.07) | *** |
| *ΣPUFA n-3* | 0.17 (0.12-0.21) | 0.16 (0.10-0.21) | 0.18 (0.09-0.28) | |
| *ΣPUFA n-6* | 23.88 (21.54-26.21) | 22.55 (19.46-25.63) | 26.07 (22.51-29.63) | * |
| *ΣPUFA* | 23.62 (21.29-25.95) | 22.85 (19.79-25.91) | 26.95 (23.43-30.46) | * |

ΣPUFA: Sum of polyunsaturated fatty acid, ΣPUFA n-6 sum of omega 6 polyunsaturated fatty acid, ΣPUFA n-3: sum of omega 3 polyunsaturated fatty acid.
ΣMUFA: sum of monounsaturated fatty acid, MUFA are reported in %.

The high level of OA on these meals may be due to the use of soybean and high oleic sunflower oil in their recipes. Palmitoleic acid (C16:1), oleic acid (C18:1, cis-9), and elaidic acid (C18:1, trans-9) were the second, third, and fourth most abundant MUFA in the meat & poultry category, respectively. Values are aligned with those reported previously (Haak, De Smet, Fremaut, Walleghem, & Raes, 2008; Karakok, OzogulL, Saler, & Ozogul, 2010) and with the information reported in their nutritional labels. Even though the majority of the MUFA reported in the USDA database were detected in our samples, some of them were present in small quantities in the USDA database and not detected in our samples and vice versa. Moreover, French fries from two different franchises showed higher MUFA percentage compared to the other FA groups. Twelve UPFs containing beef as their major component showed the highest fat content among the meat & poultry category. (See Table 3-4) This abundant presence of fat in beef is also observed in other food items such as roasted rib (style: large end) which contains 26 g per serving size (3 oz) (USDA, 2019a), and in ground beef which can contain a maximum 30% fat allowed by the USDA (USDA, 2016). Previous studies in beef have reported SFA and MUFA as their dominating FAME group (Karakok et al., 2010; Wood, 1996). Beef with vegetables from an FF restaurant, reported a saturated fat content of 21.4% in their website, which is slightly lower than our 26.94% of SFA, this confirms that our results could be more accurate.

Baby foods are RTE meals with the highest fat and MUFA contents, which are required to fulfill the infants' growing needs. Main fat sources for baby foods are meat and poultry, chicken broth and canola oil.

MUFA was dominated by oleic acid (C18:1, cis-9), palmitoleic acid (C16:1), and 11-eicosenoic acid (C20:1). PUFA in baby foods was led by linolenic acid, LA (C18:2cis), and γ-

linolenic acid (C18:2 n-6), GLA (C18:3n6). Lastly, 3 trans FAs were detected in our samples: elaidic acid (C18:1,trans-9), palmitelaidic acid (C16:1trans) and rumenic acid (C18:2trans), with elaidic acid (C18:1,trans-9) being the trans FA with the highest amount.

### 3.3.5 Polyunsaturated Fatty Acids (PUFA)

Dietary fat is directly related with the use of vegetables oils and other ingredients such a fish, avocado, among others, which contain high PUFA levels. PUFA was the third most abundant group in all UPFs with no significant differences between FF and RTE. Linoleic acid (C18:2 n-6, LA) was the most abundant followed by α-linolenic acid (C18:3 n-3, LNA). The egg & egg's derivatives category has the highest content of LA followed by seafood. These results agree with literature (Bemrah, Sirot, Leblanc, & Volatier, 2009) Simopoulos & Salem, 1992). The main ingredient of macaroni salad is mayonnaise, and both have similar fatty acid profile in agreement with those reported in literature (Tavella et al., 2000). Therefore, PUFA was also the predominant group in macaroni salad and traces presence of trans fatty acid (18:2, trans-9,12) was detected just in mayonnaise.

Less than 1% of the total fat contained trans fatty acids. Results agree with federal regulations about the total content of trans fatty acid in foodstuff (Gonçalves Albuquerque, Santos, Silva, Oliveira, & Costa, 2018). Higher PUFA content was found in salad dressing, where results agree with previous studies that reported similar values (Jacobsen, 2015; Let, Jacobsen, & Meyer, 2007). It is worth to mention that the effect of cooking method on FA profiles, for example Mac & Cheese meals were cooked using 2 different cooking methods (boiled and microwaved, Table 3-1). No reference values were found both types of cooking methods employed in this study, however the major ingredient on these meals is cheddar

*Table 3-4: Unpublished results of the fatty acid profile of UPFs by foo category (mean and range)*

| *SFA* | Meat & Poultry (n=24) | Dairy (n=9) | Eggs & Egg products (n= 2) | Seafood (n=3) | Baby Foods (n=13) | Others (n=12) | *p*-value¶ |
|---|---|---|---|---|---|---|---|
| *C8:0* | 0.42 (0.32-0.52) | 1.05 (0.97-1.12) | 0.15 (0.06-0.24) | ND | 2.43 (0.07-4.79) | 2.05 (0.04-3.69) | **** |
| *C10:0* | 1.19 (0.96-0.14) | 2.62 (2.34-2.90) | 0.05 (0.04-0.07) d | 0.79 (-0.13-0.17) | 2.50 (1.83-3.16) | 1.55 (0.59-2.51) | **** |
| *C11:0* | 0.04 (0.02-0.05) | 0.07 (0.06-0.09) | ND | ND | 0.07 (0.05-0.10) | ND | *** |
| *C12:0* | 1.02 (0.77-1.28) | 4.36 (3.38-5.35) | 0.03 (0.01-0.04) | 1.03 (-0.07-0.21) | 1.25 (0.72-0.18) | 2.93 (1.51-4.34) | **** |
| *C14:0* | 2.61 (2.10-3.13) | 1.06 (0.98-1.15) | 0.09 (0.08-0.10) | 1.61 (0.10-0.31) | 3.29 (2.10-4.49) | 1.52 (0.97-2.08) | **** |
| *C15:0* | 0.37 (0.31-0.43) | 1.02 (0.86-1.18) | ND | 0.18 (0.05-0.30) | 0.43 (0.29-0.58) | 0.11 (0.06-0.15) | **** |
| *C16:0* | 20.77 (19.67-21.87) | 34.29 (33.57-35.01) | 10.99 (10.92-11.07) | 13.63 (12.02-15.24) | 20.32 (17.76-22.87) | 19.80 (16.31-23.30) | **** |
| *C17:0* | 0.44 (0.36-0.51) | 0.50 (0.42-0.57) | 0.09 (0.08-0.10) | 0.19 (0.14-0.24) | 0.33 (0.27-0.40) | 0.12 (0.10-0.14) | **** |
| *C18:0* | 8.43 (7.60-9.27) | 11.37 (10.19-12.55) | 4.08 (4.01-4.15) | 4.99 (4.59-5.39) | 6.78 (5.83-7.74) | 5.32 (4.68-5.96) | **** |
| *C20:0* | 0.28 (0.23-0.34) | 0.17 (0.12-0.22) | 0.29 (0.27-0.31) | 0.37 (0.33-0.41) | 0.29 (0.23-0.36) | 0.35 (0.30-0.39) | ** |
| *C21:0* | 0.11 (0.05-0.17) | ND | ND | ND | ND | ND | - |
| *C22:0* | 0.39 (0.19-0.59) | 0.17 (0.16-0.19) | 0.29 (0.24-0.34) | 0.30 (0.23-0.37) | 0.17 (0.11-0.23) | 0.22 (0.18-0.27) | - |
| *C23:0* | 0.02 (0.02-0.03) | 0.08 (0.02-0.14) | ND | ND | 0.03 (-0.08-0.14) | 0.04 (0.02-0.05) | - |
| *C24:0* | 0.31 (0.09-0.54) | 0.13 (0.11-0.14) | ND | 0.11 (0.11-0.12) | 0.25 (0.02-0.47) | 0.12 (0.10-0.15) | - |
| *ΣSFA* | 34.33 (31.13-36.53) | 66.06 (64.23-67.89) | 16.07 (15.91-16.22) | 22.23 (17.99-26.46) | 33.64 (28.07-39.21) | 30.71 (25.63-35.79) | **** |
| *MUFA* | Meat & Poultry | Dairy | Eggs & Egg products | Seafood | Baby Foods | Others | *p*-value |
| *C14:1* | 0.49 (0.39-0.60) | 0.94 (0.89-0.99) | ND | 0.48 (-0.45-1.43) | 0.53 (0.38-0.67) | 0.17 (0.07-0.27) | **** |
| *C15:1* | ND | ND | ND | ND | 0.18 (0.12-0.25) | ND | - |
| *C16:1* | 2.49 (2.19-2.79) | 1.33 (1.06-1.59) | 0.12 (0.11-0.13) | 0.75 (0.15-1.36) | 2.25 (1.78-2.72) | 0.43 (0.28-0.58) | **** |
| *C17:1* | 0.37 (0.27-0.48) | 0.27 (0.18-0.36) | 0.04 (0.02-0.07) | 0.15 (0.03-0.27) | 0.26 (0.19-0.34) | 0.06 (0.05-0.07) | **** |
| *tC18:1* | 1.44 (0.82-2.06) | ND | ND | ND | 0.14 (-0.09-0.36) | 1.04 (0.19-1.89) | - |
| *C18:1 n-9* | 39.83 (38.26-41.40) | 25.86 (24.89-26.83) | 21.41 (21.38-21.44) | 30.21 (24.22-36.19) | 40.30 (36.99-43.61) | 33.58 (29.69-37.47) | **** |
| *C20:1* | 0.49 (0.43-0.55) | 0.09 (0.07-0.10) | 0.15 (0.14-0.16) | 0.29 (0.16-0.43) | 0.49 (0.39-0.59) | 0.32 (0.23-0.41) | **** |
| *C22:1* | 0.06 (0.02-0.09) | ND | ND | 0.21 (0.16-0.26) | 0.12 (0.07-0.18) | ND | - |
| *C24:1* | 0.03 (0.02-0.07) | 0.17 (0.13-0.20) | ND | 0.52 (0.34-0.71) | 0. 13 (0.07-0.26) | ND | *** |

Table 3-4 (cont'd)

| *MUFA* | Meat & Poultry | Dairy | Eggs & Egg products | Seafood | Baby Foods | Others | *p*-value |
|---|---|---|---|---|---|---|---|
| *ΣMUFA* | 44.00 (42.29-45.71) | 28.31 (27.53-28.82) | 21.73 (21.67-29.10) | 31.37 (25.02-37.71) | 43.71 (40.25-47.17) | 34.67 (30.65-38.69) | **** |
| *PUFA* | Meat & Poultry | Dairy | Eggs & Egg products | Seafood | Baby Foods | Others | *p*-value |
| *tC18:2* | 0.34 (0.27-0.41) | 0.53 (0.42-0.64) | 0.04 (0.03-0.05) | ND | 0.40 (0.30-0.49) | 0.14 (0.07-0.29) | **** |
| *C18:2 n-6* | 18.42 (16.33-20.51) | 4.40 (3.06-5.74) | 54.95 (54.81-55.09) | 40.57 (33.29-47.84) | 18.86 (15.84-21.87) | 31.22 (25.86 (36.58) | **** |
| *C18:3n-6* | 0.12 (0.10-0.15) | 0.04 (0.04-0.05) | ND | ND | 0.10 (0.05-0.15) | 0.10 (0.06-0.13) | - |
| *C18:3n-3* | 2.12 (1.81-2.44) | 0.52 (0.43-0.62) | 7.13 (7.08-7.19) | 5.07 (4.20-5.93) | 3.13 (2.38-3.89) | 0.03886 (0.02912-0.0486) | **** |
| *C20:2n-6* | 0.77 (0.34-0.12) | 0.01 (0.01-0.01) | 0.04 (0.03-0.04) | 0.20 (-0.27-0.67) | 0.11 (0.06-0.16) | 0.70 (0.11-1.51) | *** |
| *C20:3n-3 + C20:3n-6* | 0.13 (0.12-0.14) | 0.11 (0.09-0.13) | 0.03 (0.02-0.04) | 0.03 (0.02-0.05) | 0.16 (0.09-0.23) | 0.04 (0.01-0.06) | *** |
| *C20:4n-6* | 0.34 (0.28-0.39) | 0.21 (0.18-0.24) | 0.03 (0.02-0.04) | 0.58 (-0.60-1.76) | 0. 33 (0.21-0.46) | 0.08 (0.05-0.10) | - |
| *C20:5n-3* | 0.42 (0.20-0.63) | ND | ND | ND | 0.15 (0.08-0.23) | 0.07 (0.03-0.17) | - |
| *C22:2n-6* | 0.13 (-0.13-0.39) | ND | ND | ND | 0.02 (-0.01-0.04) | 0.03 (0.03-0.06) | - |
| *C22:6n-3* | 0.05 (0.03-0.07) | 0.02 (0.02-0.03) | ND | ND | 0.06 (0.03-0.08) | 0.09 (0.05-0.13) | * |
| *ΣPUFA* | 21.67 (19.37-23.98) | 5.63 (4.21-7.04) | 62.21 (62.02-62.40) | 46.41 (38.80-54.02) | 22.65 (19.28-26.02) | 35.57 (29.35-41.79) | ** |

ΣSFA: sum of saturated fatty acid, Σ sum of MUFA: monounsaturated fatty acid, Σ Sum of PUFA: Polyunsaturated fatty acid. SFA, MUFA and PUFA are reported in %. ¶Independent-samples Kruskal-Wallis test.

cheese which has been previously reported (Manuelian et al., 2017) and used for comparison purposes of this RTE. The microwaved sample showed a high SFA content while the boiled sample showed PUFAs as its higher content, demonstrating the effect of temperature variability on food nutritional content.

Seafoods are well known to be a great source of LCFA, especially omega 3 long-chain PUFA (Bemrah et al., 2009; Morais Júnior et al., 2017; Neff, Bhavsar, Braekevelt, & Arts, 2014; Oje et al., 2004; Sirot, 2008). LC n−3 FA are primarily found in organisms that live in cold water. These organisms are especially dependent on the physicochemical properties of the FA. This is due to the high number of double bonds in the molecule lowering the melting point of the compounds, which means that, even at low temperatures, biological structures retain the fluidity necessary for life processes. Sources of LC n−3 FA with greatest nutritional significance for humans are cold-water fish, such as salmon, mackerel, herring, and tuna. The USDA recommends fish consumption of at least 8 oz (227 g) seafood per week in individuals two years old and older (Kantor, 2016). Seafood can be found in food retail (56%) and restaurants (31%); being shrimp, clamps, salmon, cod, and Alaskan Pollock the most consumed seafood among US (Love et al., 2020).

Contrary to what was expected, PUFA was the highest percentage in 2 of 3 of the seafood meals tested in this study. Some incongruencies were found after comparing certain PUFAs to previous literature values (Awogbemi, Onuh, & Inambao, 2019; Czech, Grela, & Ognik, 2015; Ekiz & Oz, 2019; Katan et al., 2020; Modzelewska-Kapituła, Pietrzak-Fiećko, Tkacz, Draszanowska, & Więk, 2019; Rant et al., 2019; Shan et al., 2019). For example, in fried shrimp from an FF restaurant, no C22:6 was detected opposed to what its reference values state; and instead of C18:3n3, we were able to identify just C18:3n6

(Czech et al., 2015). Moreover, for the fish sandwich from another FF restaurant, PUFA was not the dominating FA group as stated in references because of the significant abundance of C18:2cis in our sample compared to the higher presence of C18:1cis reported by the USDA (USDA, 2020). Results from RTE clam chowder soup are aligned with its reference values, however, a difference of 55.91% between PUFA and MUFA is reported in the USDA while our sample shows only a 1.05% difference. This suggest that either some PUFAs could be missing or that our sample contains higher amount of MUFAs. RTE soups like the clam chowder is a canned product that usually needs a reconstitution and warming steps before consumption. Differences during homemade preparation such as temperature changes, delay in warming, use of microwave vs boiling, etc. may increase the lipid oxidation process promoting autooxidation of lipid molecules which in turn, alters the overall fatty acid profile of the food item.

Differences in the PUFA content were observed between biscuits from two FF franchises. For example, EPA was present in 08-FF instead of ALA which was seen in 06-FF (APPENDIX C). This could be the result of the use of milk as part of the ingredients in 08-FF recipe which contains small amounts of EPA (USDA, 2019d), in addition to cross contamination of this FA using the same equipment for cooking procedure of different food meals which could contain EPA. Lastly, even though 4 trans FAs were reported by USDA, no trans FA was detected in 08-FF. Overall, FA profile was similar to the one reported in the USDA database, and previous studies (Amrutha Kala, 2014; KFC, 2020; McDonald's, 2017; Rutkowska, Adamska, Sinkiewicz, & Białek, 2012; B. Smith, 1985), even though traces of trans FAs were reported by the USDA for French fries, none was detected in our sample. It is noteworthy to mention that the variability in frying oils used as part of the French fries cooking procedure in these 2 FF restaurants, may result in the presence of elaidic acid in item 09-FF, and its

absence in O5-FF. As Smith and co-workers explained in their study (Song et al., 2015) the use of hydrogenated soybean oil for frying purposes caused the presence of high amounts of elaidic acid (C18:1, trans-9), while the use of an animal-vegetable mix shortening (corn oil and beef flavor) in O5-FF inhibited the formation of elaidic acid (C18:1, trans-9).

## 3.4 CONCLUSIONS

This study provides an overview of the total fat and FAME profile composition of the most popular ultra-processed foods in the US Mid-West area in US. Convenience and palatability are the main reasons to consume UPFs. However, nutritional quality and dietary patterns are jeopardized by prices and popularity of UPF meals resulting in a public health issue which should be addressed.

However, with respect to the sugar, salt, and saturated fat content, it was noticed that new challenges have arisen, especially focusing on the food industry and policy makers. Some key changes may have a great impact such as reformulation of menu items, including more vegetable and fruit options as side and/o desserts, reduction of serving size especially in beverages, sides, and desserts.

The gradual reduction of the components previously mentioned in most of the food categories studied here should become a priority. It is possible to conclude that this subject remains present and new challenges are being pointed out by several national and international organizations.

# CHAPTER 4 : CHOLESTEROL OXIDATION PRODUCTS ASSESMENT IN ULTRA-PROCESSED FOODS IN THE WESTERN DIET

## 4.1 INTRODUCTION

Dietary patterns vary around the world depending on the country's preferences and even regions within a same country. This variation is a result of differences in lifestyle and culture. Interestingly, these dietary patterns have changed over the past 60 years, changing the proportions of different nutrient intake (National Geographic, 2011). The importance of a good diet relies in the energy and nutrients obtained by the consumed food to improve overall health (National Institute of Diabetes and Digestive and Kindey Diseases, 2019). The US National Institute of Diabetes and Digestive and Kidney Diseases recommends seeking nutrition resources to help manage different health conditions such as diabetes, obesity, and kidney disease.

Unfortunately, not all diets contain the necessary nutrients to maintain healthy dietary patterns resulting in a cumulative detrimental effect in people's health (Office of Disease Prevention and Health Promotion, 2015). A combination of these deficits and the excessive consumption of refined grains, processed meats, sodium, sugars, and trans-fat, creates the perfect scheme for the development of the current "pandemic of chronic diseases" (Mozaffarian, 2011). Changes in lifestyle behavior have significantly contributed to the rise of rates of non-communicable diseases, which are also known as chronic diet-related diseases (Office of Disease Prevention and Health Promotion, 2015). Cardiovascular diseases, diabetes, obesity, and cancer, are some of the examples of chronic diseases that could be caused by poor diet quality (World Health Organization, 2003).

In fact, the 2015-2020 Dietary Guidelines states that 117 million Americans have one or more preventable chronic disease, which many relating to poor quality diet. Moreover, overweight and obesity rates are steadily increasing, resulting in extremely high cost health treatments, reaching $245 billion for diabetes costs in 2012 alone (Office of Disease Prevention and Health Promotion, 2015). The American diet, also known as the Western diet, is a high fat, animal protein and sugar one, with very low levels of dietary fiber, vegetables, and fruits (Chiba et al., 2019; Park et al., 2011; Statovci et al., 2017; Tilg & Moschen, 2015; Uranga et al., 2016). Therefore, it is crucial to perform accurate and reliable dietary assessments for different types of food items present in the Western diet that will help elucidate the association between these food items and disease risk (Yin et al., 2017). Different dietary markers of exposure for many foods have been putatively identified (Cross et al., 2011; Gibbons et al., 2015; K. A. Guertin et al., 2015; Kristin A Guertin et al., 2014; Heinzmann et al., 2010; Jenab et al., 2009; O'Gorman et al., 2013; Yin et al., 2017). However, many more food items which are a significant part of Western diet, still need to be analyzed to identify potential biomarkers that will help us recognize and potentially prevent, the development of chronic diseases in the US population.

### 4.1.1 Western Diet

The Western diet is commonly described as one with high fat and refined sugars contents. Additionally, it is characterized by a high intake of red and processed meats, sweets, fried foods, refined grains, and overuse of salt (Drewnowski et al., 2013; Drewnowski & Rehm, 2013; Francis & Stevenson, 2013; Hintze et al., 2012; Myles, 2014). The "*Western diet*" term has been associated and adopted as a term to describe the modern standard American diet. It has been observed in different studies that the

components of the Western diet lack of nutritional value, and are micronutrient deficient (Hintze et al., 2012; Myles, 2014). This intake deficit of micronutrients results in levels below the Recommended Daily Allowances (RDAs) and an excess in consumption of energy-dense and nutrient-poor foods (Hintze, Benninghoff, & Ward, 2012). Studies have shown that 92% of meals in the US exceeded typical energy requirements for a single eating occasion (Urban et al., 2016). Restaurant foods, which are popular in the Western diet, tend to have large portion sizes as well as high energy density (McCrory et al., 1999; Urban et al., 2011), which has been causally associated with higher energy intake. (Diliberti et al., 2004; Rolls et al., 1999, 2005, 2006). According to NHANES 2005-2006, 68% of the top 25 sources of calories among Americans 2 years and older, were high-energy food items (U.S. Department of Agriculture & U.S. Department of Health and Human Services, 2010).

The existence of all these energy-dense food items has been a result of significant changes that global food systems have undergone, resulting in advanced food processing techniques to increase products availability, affordability and marketing of highly processed foods (Floros et al., 2010; B. A. Swinburn et al., 2011; Boyd A Swinburn et al., 2011). Because of the high demand of food supplies and the dietary patterns of consumption of packed, branded, ready to eat, and ready to drink products, these “convenient” foods have been created, using processing conditions that alter their structure, nutritional content, and taste (van Boekel et al., 2010; van Trijp & Ingenbleek, 2010; Wahlqvist, 2016; Zobel et al., 2016). These food products are known as “processed foods” and “ultra-processed foods” because of the different processing techniques and methods that they undergo as part of their preparation for sale to the public. Processed and ultra-processed foods (which differ in the number of processing techniques applied during their production) are the predominant items in modern societies, hence, the heterogeneity of food processing advocates for new

classification tools that can facilitate the assessment of food processing on nutritional quality and health.

### 4.1.2 Ultra-Processed Foods and NOVA Classification

Ultra-processed food is characterized to be inexpensive, *highly processed,* rich in calories, but low in some essential micronutrients such as mineral and vitamins. Nearly 60% of the calories consumed by the average Americans come from "ultra-processed" foods (Martínez Steele et al., 2016). During the past 40 years, an increase of 217 to 491 kcal/day per capita food consumption and self-reported energy intake has been reported (Health, 2011; USDA Economic Research Service, 2014). Studies have shown that ultra-processed foods comprise 57.9% of energy intake (Martínez Steele et al., 2016). These meals are mostly consumed away from home and have been proposed as a major contributor to rising energy intake (Urban et al., 2016).

The consumption of ultra-processed, calorie-dense, and nutrition-poor food, especially those items from the Western diet, is increasing in developed countries (Juul et al., 2018; Setyowati et al., 2018). The urban lifestyle with limited time, work overload, and busy schedules influences individuals to buy the most convenient, fast, and cheapest meal options. Therefore, prepared, pre-processed, and ready to eat meals are the preferred ones by the urban population. Nearly every food product present in the Western diet is considered a processed food product. Processing itself does not represent a threat to human health. On the contrary, different processes have been focused to ensure food safety and microbial reduction. However, the formation of harmful compounds derived from different processing parameters requires more attention during the manufacturing of these food products.

Therefore, processing methods play a crucial role in the evaluation of the overall chemical safety of the Western diet.

Ultra-processed foods undergo, at least, 4 different process steps promoting the formation of molecules such as COPs. Thus, a new food classification method such as NOVA (not an acronym) classification that considers the processing conditions of each food product is needed to facilitate the study and understanding of these ultra-processed foods. NOVA classification is defined as "the food classification that categorizes foods according to the extent and purpose of food processing, rather than in terms of nutrients" (Juul et al., 2018; Martínez Steele et al., 2016; Moubarac et al., 2017) and is recognized by the Food and Agriculture Organization (FAO) and Pan-American Health Organization (PAHO) as a valid tool for nutrition and public health research (C. Monteiro et al., 2016; C. A. Monteiro et al., 2019). NOVA groups foods according to the nature, extent and purpose of the industrial processing they undergo (Moubarac et al., 2017).

NOVA classifies foods and food products into 4 different groups (Figure 4-1). Following NOVA's definition, Group 4 - Ultra-processed foods (UPF) is comprised of "industrial formulations typically with five or more and usually many ingredients" (C. Monteiro et al., 2016). Ingredients found only in ultra-processed products include substances not commonly used in culinary preparations, and additives whose purpose is to imitate sensory qualities of group 1 foods or of culinary preparations of these foods, or to disguise undesirable sensory qualities of the final product. Fast foods (including small, large, and non-chain restaurants) and Ready to eat (RTE) products are also included in this group. The term fast food has been defined as "easily prepared processed food served in snack bars and restaurants as a quick meal or to be taken away" ("Definition of fast foods," 2017). A suitable definition could be

the sale of mass-produced UPF characterized by its “fast” and “convenient” preparation time. RTE are products previously processed and packed for sale which require minimum preparation effort, making them a suitable option for people looking for a “quick” and “easier” meal.

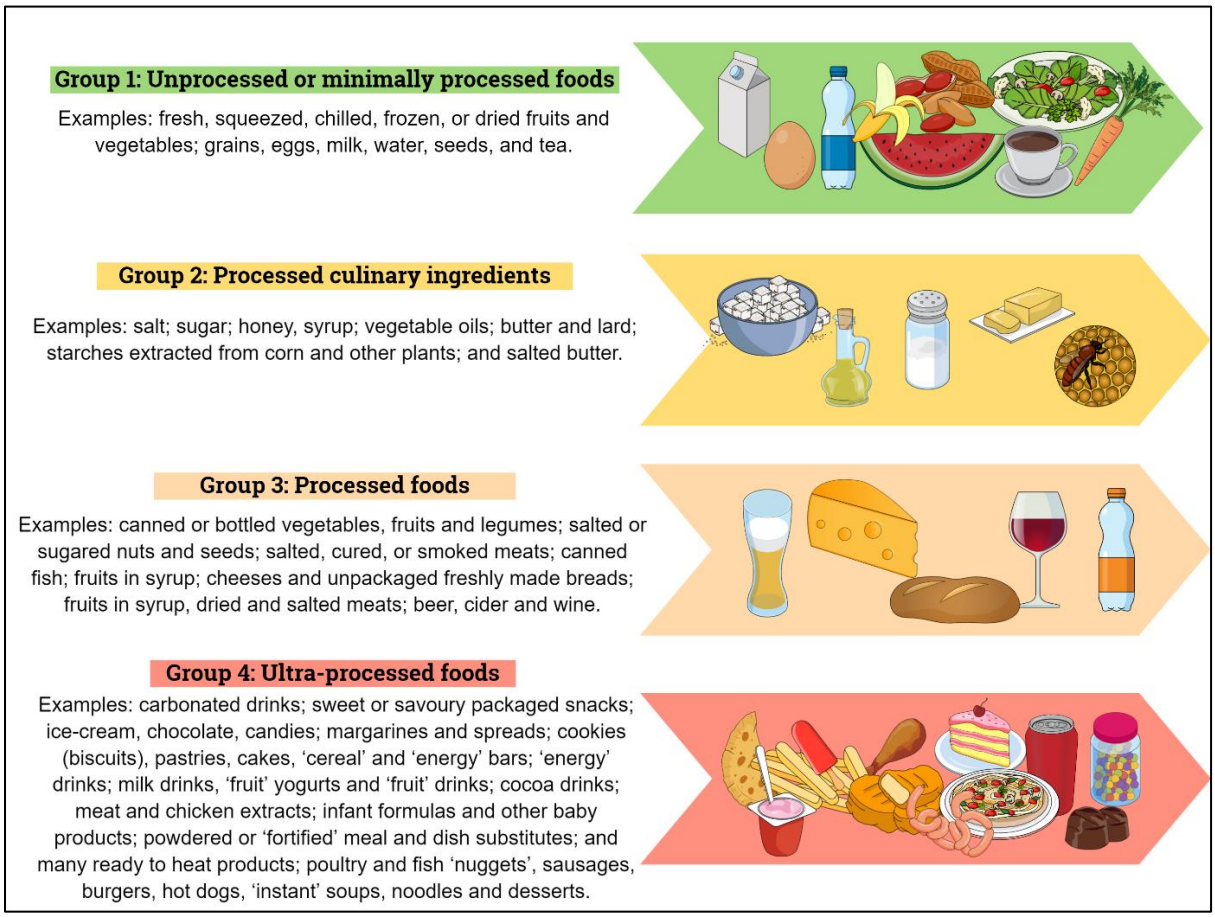


***Figure 4-1:*** *NOVA food classification based on food processing*

The main concern related with the high consumption of UPF relies on food safety. Even tough industrial food processes are generally designed to improve food quality, nutrition, and safety, processing can lead to unintentional accumulation of substances such as COPs, which are formed because of thermal treatments, light exposure, storage, and aging of food, and may therefore also negatively impact overall safety.

### 4.1.3 COPs as Biomarkers

A wide range of degradative compounds, such as aldehydes, ketones, and peroxides, are produced because of cholesterol oxidation. These compounds are known as cholesterol oxidation products (COPs) and have been studied because of their potential relationship with different chronic diseases. Maldonado and co-workers (2018) showed that there is a significant amount of evidence that COPs exert biological and pathological activities in both in vitro and in vivo systems, with potential health concerns for humans.

As it was previously mentioned in Chapter 1, Lee et al., 2009 have suggested that the apparent association of specific COPs with people more prone to suffer of these chronic diseases could be beneficial, as COPs could be used as biomarkers for these diseases (Aldini et al., 2010; Alkazemi et al., 2008; Iuliano et al., 2003).

7-Keto has been largely detected and quantified in both model and food systems such as infant formula, suggesting it as one of the most reliable biomarker of cholesterol oxidation (A. Kilvington, Barnaba, Rajasekaran, Laurens Leimanis, & Medina-Meza, 2021; Rodriguez-Estrada et al., 2014). However, processing methods such as ultra-high temperature (UHT) pasteurization can also generate sidechain COPs (Pikul et al., 2013), including the demonstrated cytotoxic 25-OH. It is still unclear if COPs' composition can reflect specific processing technologies, as some early studies have demonstrated. Therefore, more mechanistic, and quantitative studies for a suitable estimation of COPs in food products, and a harmonization of methodologies for their determination is still needed.

### 4.1.4 Effect of Food Processing on Cholesterol Oxidation

Temperature and pressure changes, as well as exposure to light applied during processing, and even packaging and storage techniques, are some factors that promote COP formation. Industrial processing (i.e., cooking, pasteurization, canning, etc.) occurs at variable times and temperatures depending on the type of process and food (Maldonado-Pereira et al., 2018). Food processing temperatures range between 121°C (canning of meats) and 400°C (spray drying-used in the manufacturing of infant formulas) (Meister et al., n.d.; Verardo et al., 2017), and even the US Department of Health and Human Services, suggests that meat and poultry should be cooked at a minimum of 162 °C (FoodSafety, 2017). Moreover, studies show that several cooking methods considerably increase the formation of COPs. Microwave heating (Herzallah, 2005), pan roasting, oven grilling (Khan et al., 2015), oil-frying (Dorota Derewiaka & Obiedziński, 2010) and other forms of cooking (Nielsen et al., 1996) affect the production of COPs.

Until today, there is no existent database of these compound in the US diet. A COP database of the Western Diet that will allow us to identify potential biomarkers of thermal degradation in UPFs will be reported in this chapter. Eventually, this database will help to develop a nationwide framework of tools for surveillance, inspection, and traceability of COPs in UPF. It will enable knowledge about processing-formed unintentional compounds which will help to improve or even, avoid processing methods to ensure food safety and therefore, wellbeing of the US population.

## 4.2 MATERIALS AND METHODS

A total of 63 food items were analyzed in this study. A general overview of the materials used and methodology for the analysis of UPF is provided below. Details for each sample analysis as well as brand information can be found in the Appendixes C and D.

### 4.2.1 Materials, Chemicals, and Reagents

Methanol was from Sigma-Aldrich (St. Louis, MO). Chloroform was obtained from Omni Solv (Burlington, MA), hexane was purchased from VWR BDH Chemicals (Batavia, IL), 1-butanol and potassium chloride (KCl) from J. T. Baker (Allentown, PA) and diethyl ether was purchased from Fisher Chemical (Pittsburgh, PA). Sodium sulfate anhydrous ($Na_2SO_4$), Sodium chloride (NaCl) were also purchased from VWR BDH Chemicals. standards of 7$\alpha$-hydroxycholesterol (7$\alpha$-OH), 7$\beta$-hydroxycholesterol (7$\beta$-OH), 5,6$\alpha$-epoxycholesterol (5,6$\alpha$-Epoxy), 5,6$\beta$-epoxycholesterol (5,6$\beta$-Epoxy), triol, 7-ketocholesterol (7-Keto) were purchased from Steraloids (Newport, RI), and purified by using aminopropyl ($NH_2$) cartridges (500 mg/ 3 mL) from Phenomenex (Torrance, CA).

### 4.2.2 Sample Collection and Preparation

Samples were collected and prepared following the same condition and procedure explained in Maldonado-Pereira et al. 2021. Forty RTE items and 23 FF meals were selected based on the FDA's TDS inventory (FDA, 2018). Additional information regarding the sample's codes used in this study is available in Appendix C. Eight of the most popular franchisees in the state of Michigan covering more than 75% of the national market (Dunford et al., 2017; Powell et al., 2019; Rwithley, 2019; Tran et al., 2019). In addition, food items and meals were grouped in subcategories according to the fat source as follow:

eggs and egg's derivatives (E), dairy products (D), meat and poultry (MP), seafood (S), baby food (BF). Additional food items that did not fit in any of the previous categories, such as potato-products (potato crisps with and without added flavors, French fries from restaurants and takeaway, frozen potatoes pre-fried and fried, and homemade French fries), pasta, salad dressings, and popcorn (sweet or salty) were grouped as other products (O). Once the UPF arrived at the laboratory, an excel form was filled out with the following information: (1) UPF's name; (2) price; (3) place of purchase, date, and time of collection; (4) type of food (RTE or FF); (5) nutritional declaration (energy, fat, saturated fatty acids, carbohydrates, sugars, fiber, protein, and salt); (6) portion size; (7) list of ingredients; (8) expiring date; and (9) other relevant information.

### 4.2.3 Sample Preparation

FF meals were purchased from each individual franchise and brought to the laboratory for immediate analysis. RTE meals were purchased from different local supermarket stores, and immediately brought to the laboratory. Storage conditions were followed according to the label instructions (fresh foods were kept in a fridge at 4 °C and frozen meals were kept at -20 °C or the temperature indicated in the label). All UPFs were analyzed before the expiration date. When further cooking procedures were required, items were prepared in accordance with manufacturer's instructions. Kitchen equipment available at the Michigan State University's Food Science Laboratory was supplied and used for this study. All the samples were homogenized using an Ultra-Turrax® (Tekmar TP 18/10S1 Cincinnati, OH) for 3 min at 5000 rpm, split and stored accordingly, depending on the food matrix.

### 4.2.4 Lipid Extraction

Lipid fraction was extracted according to Folch and coworkers (Folch et al., 1957) cold extraction method with some modifications depending the food matrix. Specific details for analysis are described in the Supplementary Information-I. 30 grams of sample were minced and homogenized using an Ultratourrax with 200 mL of a chloroform:methanol solution (1:1, v/v) in a 500 mL glass bottle with screwcap. Sample was mixed for 15 min at 300 rpm. Homogenization was performed using a Ultratourrax for 3 minutes. Bottle was kept in an oven at 60 °C for 20 min before adding 100 mL chloroform. After 2 min of homogenization, the content of the bottle was filtered. The filtrate was mixed thoroughly with a 100 mL of 1 M KCl solution. Samples were left overnight at 4 °C. Then, the lower phase containing lipids was collected and dried with a vacuum evaporator (Organomation S-EVAP-RB, Berlin MA) at 25 in Hg and 60°C. Total fat content was determined gravimetrically.

### 4.2.5 Thiobarbituric Acid Reactive Substances (TBARS)

The method modified by Miller et al (1994), was used to measure lipid oxidation in all UP foods. Some additional modifications were performed to the protocol. 60 mg of fat were weighed into a 10 mL glass test tube with screw cap and the following reagents were added: 100 μL BHT solution (0.2 mg/mL in water) and 4.9 mL extracting solution (10% TCA in 0.l M $H_2PO_3$). Each sample was vigorously vortexed in the test tube. Sample blanks were analyzed for each sample. A standard curve was prepared using 0-5 mL TEP solution (10 μM). Test tubes were incubated in the dark for 15-17 hr. at room temperature (25°C). TBARS were expressed as μg malondialdehyde (MDA)/g fat sample (dry weight). No adjustment of total volume was needed since fat sample did not contain moisture.

### 4.2.6 Total Cholesterol and Phytosterols Content

It is important to mention that the FHEL has developed *a simultaneous quantification method* for both compound groups: Cholesterol and phytosterols (Alice Kilvington, Maldonado-Pereira, Torres-Palacios, & Medina-Meza, 2019). Cold saponification at room temperature was performed following Bonoli's protocol with some modifications (Bonoli et al., 2008). Two hundred mg of fat were placed into a glass tube and dried under a nitrogen stream. 40 ug of 5α-cholestane (5α) and 50 ug of 19-hydroxycholesterol (19-OH) were added as internal standards. Ten mL of 1 N methanolic KOH were added and vortexed. The unsaponifiable fraction was collected and sodium sulfate was added. The mixture was left standing for 2 h and then filtered over a bed of sodium sulfate and collected in a round bottom flask. The solution was then dried in a vacuum evaporator. The sterols were then collected in diethyl ether and dried with a stream of nitrogen. One mL of 4:1 hexane: isopropanol was added and then stored at − 20 ◦C until analysis. One-hundred microliters (µL) were derivatized by drying with nitrogen and adding 100 µL of pyridine and 100 µL of silanization solution and then heated at 60 ◦C for 40 min. The solution was then dried and resuspended in 1 mL hexane. Two µL were injected into a GC coupled to a MS (GC-MS) (Shimadzu GCMS-QP 2010 SE, LabSolutions GCMS Solution Version 4.45). Injector temperature was set at 320 ◦C. The oven temperature profile was programmed to start at 260 ◦C and increase to 300 ◦C at a rate of 2.5 ◦C/ min, then from 300 ◦C to 320 ◦C at a rate of 8 ◦C/min and held for 1 min. Helium was used as a carrier gas with a pressure of 134 kPa. The sterol identification was done by comparing retention times and mass fragmentations from pure standards. Quantification was done by comparing peak areas using the internal standards.

### 4.2.7 COPs Quantification

The remaining 900 µL of the unsaponifiable fat were enriched in the sterol oxidation products by solid-phase extraction (SPE), using NH2 Strata® SPE cartridge. Their purification by $NH_2$-SPE was performed according to Rose-Sallin, Huggett, Bosset, Tabacchi & Fay, with slightly modifications. Samples were diluted in 500 µL of n-hexane:ethyl acetate (95:5, v/v), followed by the activation of the SPE column by adding 3 mL of n-hexane and 2-3 mm of sodium sulfate, and allowed to settle in the column. Enriched samples were dried using a nitrogen stream and then derivatized as described above. One µL of the sample were injected into a GC coupled to a MS (GC-MS) (Shimadzu GCMS-QP 2010 SE, LabSolutions GCMS Solution Version 4.45). The injector temperature was set to 320 ◦C. The oven temperature profile was programmed from 260 ◦C to 280 ◦C at a rate of 2 ◦C/min and held for 7 min and then from 280 ◦C to 315 ◦C at a rate of 1.5 ◦C/min. Identification of compounds was performed using retention times and mass fragmentations from commercial standards. Using the TIC method, the quantification of the total sterols content was done by comparing the peak areas to the internal standard, 19-OH. The Selected Ion Monitoring (SIM) method was used to profile the COPs in the IF. The injector temperature was set to 320 ◦C. The oven temperature profile was programmed from 260 ◦C to 280 ◦C at a rate of 2.0 ◦C/min and held for 7 min and then from 280 ◦C to 315 ◦C at a rate of 1.5 ◦C/min. Helium was used as a carrier gas with a pressure of 49.2 kPa and a column flow rate of 0.41 mL/min. The ion source temperature for the MS was set at 230 ◦C. Identification of compounds was done using retention times and mass fragmentations from commercial standards.

## 4.3 STATISTICAL ANALYSIS

Descriptive statistics were calculated overall and by category. Both mean, and confidence interval (95%) were reported. All the statistical analysis were computed using SPSS v.26 (IBM).

## 4.4 PRELIMINARY RESULTS

A set of UPFs samples together with several homecooked plates were tested at the beginning of this study to confirm the accuracy and effectiveness of this project's methodology. Over 266 food items from the Total Dietary Study (TDS)-FDA inventory (Kulig et al., 2016) into: a) High priority, products considered as essential items in every household (i.e., milk, eggs, butter, etc.), b) Ready-to-eat (RTE) food items, representing pre-cooked meals which require simple and fast cooking processes (i.e., microwaving, oven-warming etc.), and c) Fast Foods (FF), representing meals that can be purchased at FF chains. Over 150 homecooked meals (n=168, 3 reps each) were prepared following the high priority category. A total of 120 different RTE food items (n=40, 3 reps per item) were purchased from 3 of the top retail stores in the US available in the area (i.e., Walmart, Kroger, and Meijer). FF items (n=22, 3 reps each) were obtained from the top 10 most consumed FF franchises (i.e., Domino's Pizza, Marco's Pizza, McDonalds, Chipotle, Subway, Panda Express, Chick Fil' A, Jimmy Johns, and KFC) (O'Callaghan et al., 2014).

Samples were tested for total fat, cholesterol, and **eight COPs** (referred in this test as "dietary oxidative species" (DOxS)), and phytosterol contents. Secondary oxidation products were analyzed through (TBARS) assay and total SFA, MUFA, and PUFA were quantified.

### 4.4.1 Dietary Sterols' Levels

The overall dietary cholesterol varied from 48.2 mg/100 g of fat (± 5.9, 08-FF) to 1,147.7 mg/100 g of fat (± 160.8, Baby food). S3-FF contained the highest cholesterol value, followed by MP15-FF (both from the FF group). In addition, home-cooked bacon (pan-fried) showed the highest cholesterol oxidation percentage with 96% compared to the raw product.

Oven-roasted pork ham had a 64% of oxidized cholesterol and boiled frankfurter showed just 15%, which suggests a relationship between the cooking method and the oxidation percentage, involving different factors such as temperature conditions and the food matrix. Even though cholesterol was the most abundant sterol in these samples, stigmasterol and campesterol were also identified and quantified. 07-FF and MP14-FF contained the highest amounts of stigmasterol (224.87 and 209.33 mg/100g fat, respectively). Campesterol quantities were led by 05-FF and 09-FF (192.00 and 184.08 mg/100 g fat, respectively; and even quantities of brassicasterol were detected in 09-FF and MP17-FF (44.19 and 27.56 mg/100 g fat, respectively). In addition, MP3-RTE showed the highest stigmasterol content with 259.84 mg/100g fat.

Results confirm that sterols oxidation are triggered by light (Maerker & Jones, 1993b; I. G. Medina-Meza & C. Barnaba, 2013; Ilce Gabriela Medina-Meza et al., 2012), heat (Chien et al., 1998; D. Derewiaka & Molinska nee Sosinska, 2015; Tai, Chen, & Chen, 1999), radiation (Maerker & Jones, 1993a; L. L. Smith, 1996), metal ions (Hur et al., 2007a; Jo et al., 1999), and other agents that have the ability to lower the energetic requirements for the reaction to occur (Beltran et al., 2004; I. G. Medina-Meza & C. Barnaba, 2013).

### 4.4.2 Dietary Oxidized Sterols DOxS

As expected, the concentration of DOSs was positively associated with the cholesterol concentration (Figure 4-2). However, the association was far from perfect (correlation=0.504) indicating that food composition and food processing affects the formation of DOxS from cholesterol.

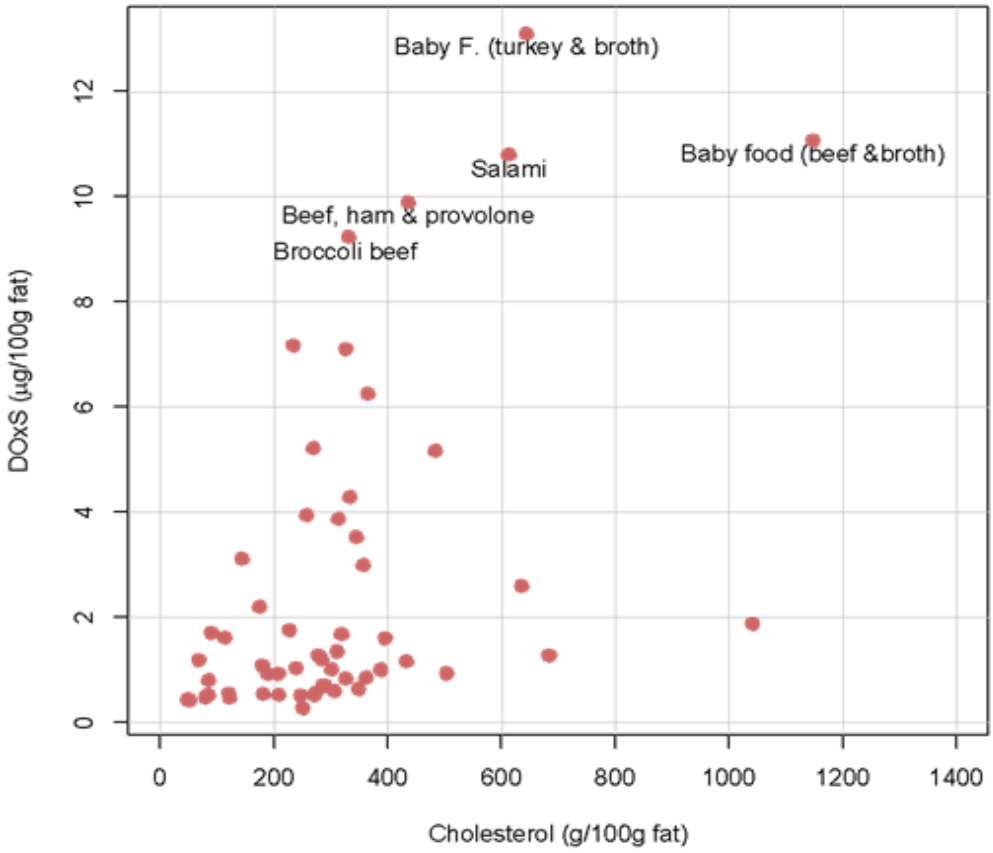


***Figure 4-2:*** *Cholesterol and oxidized sterols*

Among the detected DOxS derived from cholesterol, 7α-OH and 7β-OH were the most abundant ones; however, in some highly processed food items, 7-keto was present in high concentration. This variability suggests a relationship between DOxS and food matrix. On the other hand, among the detected DOxS derived from phytosterols, β-sitosterol, oxidized

campesterol and stigmasterol derivatives were the most abundant ones. However, oxidized β-sitosterol was the dominant phytosterol within RTE items.

Our protocol detected more than 30 oxidized metabolites. We isolated and quantified the most abundant DOxS in all food products (7α-OH, 7β-OH, 5,6α-Epoxy, 5,6β-Epoxy, Triol, 7-Keto, and 24-methylenecholesterol, and 6-Keto). Using mixed-effects models we estimated the proportion of variance of each of the DOxS explained by food type, category and food item.

### 4.4.3 Multivariate Analysis of DOxS

A multivariate analysis of the lipid fingerprinting profiles revealed a clear clustering of compounds and food items–the DOxS form a clear cluster of compounds (see red square covering rows/columns 3 to 10 in Figure 4-3).

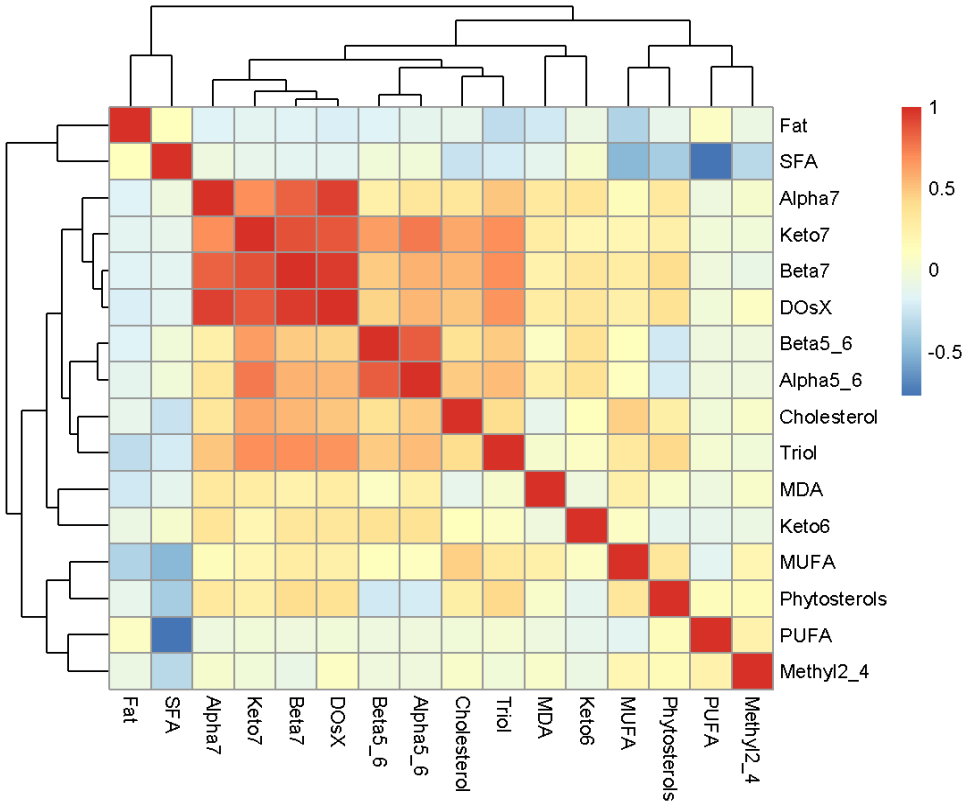


***Figure 4-3:*** *Heatmap of the correlation of lipid profiles across food meals*

Importantly, our protocol provides simultaneous quantification of both DOxS and phytosterols. For FF, other lipid oxidized derivatives were also detected such as phytosterols oxidation products (POPs). Even though cholesterol was the most abundant sterol in these samples, stigmasterol, campesterol and their oxidized derivatives were also present.

## 4.5 RESULTS FROM THE COPS ASSESSMENT OF UPFS

### 4.5.1 Fat Content in UPFs

Fat content of all 63 UPFs were previously reported in Maldonado-Pereira et al. 2021. Fat was varied between food groups ($p < 0.001$). Dairy was the food group with the highest fat content, followed by eggs and egg's derivatives, and meat and poultry (which includes chicken, beef, turkey, pork, and combinations of these meats). The majority of the UPFs showed a higher fat content compared to the values reported in either their nutritional labels or webpages.

### 4.5.2 TBARS

MDA content for FF meals and RTE items are shown in Table 4-1. Results were significantly different between food groups. Meat and poultry, dairy, eggs and egg's derivatives, and seafood showed the highest MDA contents. No presence of MDA in the sample was detected for MP12-FF, MP16-FF, MP17-FF, MP14-FF, and O8-RTE. RTE items with the lowest MDA values were: D4-RTE, D6-RTE, D2-RTE, D7-RTE, E1-RTE, D5-RTE, O4-RTE, S1-RTE, BF9-RTE, O1-RTE (0.16, 0.20, 0.36, 0.37, 0.56, 1.04, 1.24, 1.26, 1.48, and 1.88 µg MDA/g fat, respectively).

### 4.5.3 Total Cholesterol Content

Cholesterol values for FFs and RTE items are shown in Table 4-2. FF meals had the highest cholesterol contents among all UPFs. Sixteen FF meals out of 23 showed a higher cholesterol content compared to the value found in either the USDA webpage, the FF's nutritional webpages, or other Food Nutrition webpages used for this analysis (Eat this much (DeMenthon, n.d.) and Fast Food Nutrition (Lenhoff, 2005)). Between food groups, meat and poultry were the group with the highest cholesterol values.

Two items (O10-FF and O7-FF) showed cholesterol values of 90.13 and 268.72 mg/100 g fat, respectively; even when their cholesterol content in each official FF webpage stablished that these items have 0 mg/100 g fat. On the contrary, no cholesterol was detected for O5-FF and O9-FF, which aligns with the cholesterol value expressed in the USDA food database and KFC's official webpage, respectively. Lastly, no cholesterol was detected for O6-FF even though USDA food database shows a cholesterol value of 222.99 mg/100 g fat.

Nine RTE items out of 40 showed a higher cholesterol content compared to the value found in the item's nutrition fact label (598.42 $\pm$ 126.63 mg/100 g fat –MP2-RTE, 102.60 $\pm$ 18.68 mg/100 g fat – E1-RTE, 276.81 $\pm$ 1.83 mg/100 g fat – D4-RTE, 307.81 $\pm$ 3.54 mg/100 g fat – MP3-RTE, 300.53 $\pm$ 20.51 mg/100 g fat – E2-RTE, 2,135.58 $\pm$ 168.32 mg/100 g fat – MP6-RTE, 1,247 $\pm$ 227.45 mg/100 g fat – BF1-RTE, and 313.34 $\pm$ 27.54 mg/100 g fat – BF11-RTE. BF9-RTE showed a cholesterol content of 162.73 $\pm$ 95.67 mg/100 g fat when the USDA food database reported 0 mg/100 g fat. The other 30 RTE items showed a lower cholesterol content than the one expressed in each nutrition fact label.

### 4.5.4 Phytosterols and Tocopherols Quantification

Six phytosterols (Campesterol, Stigmasterol, Brassicasterol, β-Sitosterol, and Fucosterol), and β-Tocopherol were quantified for FF meals and RTE items (APPENDIX F). FF meals showed the highest phytosterol contents ($p < 0.001$), however, the abundance of each phytosterol varied between FF and RTE. β-Sitosterol, Campesterol, and Stigmasterol were the leading phytosterols between FF and RTE. However, β-Sitosterol was the phytosterol with the highest quantities within the FF meals (343.35 ± 137.26 mg/100 g fat – MP18-FF, 333.41 ± 45.98 mg/100 g fat -09-FF, 331.87 ± 34.11 mg/100 g fat – 05-FF, and 296.67 ± 38.94 mg/100 g fat – MP11-FF). Stigmasterol was the second lead phytosterol between the FF meals with 224.87 ± 30.79 mg/100 g fat – 07-FF, 209.33 ± 40.88 mg/100 g fat – MP14-FF, and 168.10 ± 24.33 mg/100 g fat – MP23-FF. Lastly, Campesterol quantites were: 192.00 ± 21.55 mg/100 g fat – 05-FF, 184.08 ± 25.17 mg/100 g fat – 09-FF, and 122.85 ± 18.26 mg/100 g fat MP11-FF.

For RTE, β-Sitosterol was also the phytosterol with the highest quantities within the RTE items (626.48 ± 57.01 mg/100 g fat – MP3-FF, 498.98 ± 129.71 mg/100 g fat – BF4-RTE, 435.62 ± 41.06 mg/100 g fat – BF11-RTE, 421.59 ± 137.27 mg/100 g fat BF7-RTE, 391.77 ± 93.78 mg/100 g fat BF5-RTE, 236.18 ± 18.19 mg/100 g fat – BF12-RTE). Campesterol was the second highest phytosterol between the RTE items with 190.88 ± 21.15 mg/100 g fat for BF7-RTE, 182.76 ± 53.20 mg/100 g fat for BF4-RTE, and 136.48 ± 47.29 mg/100 g fat BF5-RTE. Lastly, Stigmasterol was the 3rd most abundant phytosterol with values of: 259.84 ± 40.55 mg/100 g fat for MP3-RTE, 43.37 ± 12.51 mg/100 g fat for D3-RTE, and 42.06 ± 13.92 mg/100 g fat for E1-RTE.

Traces of Fucosterol were observed in O10-FF, and no presence of β-Tocopherol was observed in FF meals. β-Tocopherol was quantified in O2-RTE (0.019 ± 0.012 mg/100 g fat). Brassicasterol was not detected in any of the RTE items.

Food groups that contained ample amounts of phytosterols were pork, eggs and egg's derivatives, baby foods, and "others" ($p < 0.001$).

### 4.5.5 COPs Quantification

Twelve different COPs (7α-OH, 7β-OH, 4β-OH, 5,6α-Epoxy, 5,6β-Epoxy, triol, 7-Keto, 6-Keto, 20α-OH, 22-OH, 24-OH, and 25-OH) were determined in FF meals and RTE items by GC-MS operated in SIM mode (Table 4-3 and 4-4, respectively). Total COPs values in FF meals ranged between 0.91 and 39.13 mg/100 g fat, and from 0.14 to 63.59 mg/100 g fat between RTE. The dominant COP was 7α-OH, followed by 7β-OH. For 5,6α-Epoxy, MP21-FF (0.070 ± 0.028 mg/100 g fat) and the MP23-FF (0.040 ± 0.0019 mg/100 g fat) led the list. Moreover, 5,6β-Epoxy was detected in high amounts in MP21-FF (0.15 ± 0.049 mg/100 g fat) and O10-FF (0.15 ± 0.021 mg/100 g fat). MP15-FF was the only meal that contained all COPs resulted from the oxidation of cholesterol at carbon-position 7 (C-7). These are: 7α-OH, 7β-OH, triol, 7-Keto.

Differences between food groups were significant ($p < 0.001$), being meat and poultry the leading group. 4β-OH was only detected in FF meals and BF1-RTE was the only baby food to show content of 5,6α-Epoxy and 5,6β-Epoxy. For side-chain COPs, 25-OH and 22-OH were the most abundant ones, reaching values of 3.60 mg/100 g fat in baby food samples. D3-RTE contained traces of 25-OH.

***Table 4-1:*** *Unpublished results of MDA concentration in RTE items and FF meals*

| *Ready to Eat* | | | | | |
|---|---|---|---|---|---|
| *Food Category* | *Food Item* | *μg MDA/g fat ± STD* | *Food Category* | *Food Item* | *μg MDA/g fat ± STD* |
| Dairy | D1-RTE | 3.58 ± 1.28 | Baby food | BF1-RTE | - |
| | D2-RTE | 0.36 ± 0.23 | | BF2-RTE | - |
| | D3-RTE | - | | BF3-RTE | 19.42 ± 7.41 |
| | *D4-RTE | - | | BF4-RTE | - |
| | D5-RTE | 1.03 ± 0.052 | | BF5-RTE | 3.30 ± 0.87 |
| | D6-RTE | 0.19 ± 0.017 | | BF6-RTE | 7.95 ± 5.47 |
| | D7-RTE | 0.37 ± 0.27 | | BF7-RTE | 2.47 ± 0.42 |
| | D8-RTE | - | | BF8-RTE | 2.81 ± 1.43 |
| | D9-RTE | - | | BF9-RTE | 1.48 ± 0.24 |
| | D10-RTE | 3.22 ± 2.18 | | BF10-RTE | 9.36 ± 0.51 |
| | D11-RTE | - | | BF11-RTE | 2.51 ± 1.09 |
| Meat & Poultry | MP1-RTE | 29.66 ± 8.43 | | BF12-RTE | 17.87 ± 11.01 |
| | MP2-RTE | 12.67 ± 3.42 | | BF13-RTE | - |
| | MP3-RTE | 7.59 ± 0.23 | Eggs & egg derivatives | E1-RTE | 0.56 ± 0.042 |
| | MP4-RTE | 7.73 ± 5.87 | | E2-RTE | 1.85 ± 1.19 |
| | MP5-RTE | 2.12 ± 1.48 | Seafood | S1-RTE | 1.26 ± 0.66 |
| | MP6-RTE | - | Others | O1-RTE | 1.88 ± 1.46 |
| | MP7-RTE | - | | O2-RTE | 3.03 ± 0.89 |
| | MP8-RTE | 6.76 ± 4.83 | | O3-RTE | 3.83 ± 0.60 |
| | MP9-RTE | 23.83 ± 18.69 | | O4-RTE | 1.24 ± 0.23 |
| *Fast Food* | | | | | |
| Meat & Poultry | MP10-FF | 4.15 ± 1.14 | Seafood | S2-FF | 2.66 ± 1.25 |
| | MP11-FF | 1.35 ± 0.79 | | S3-FF | 0.69 ± 0.38 |
| | MP12-FF | - | Others | O5-FF | 7.27 ± 2.61 |
| | MP13-FF | 3.43 ± 1.70 | | O6-FF | 1.84 ± 1.64 |
| | MP14-FF | - | | O7-FF | 2.11 ± 0.90 |
| | MP15-FF | 3.64 ± 2.24 | | O8-FF | - |
| | MP16-FF | - | | O9-FF | 3.22 ± 1.86 |
| | MP17-FF | - | | O10-FF | 6.20 ± 2.40 |
| | MP18-FF | 17.01 ± 6.32 | | | |
| | MP19-FF | 2.13 ± 0.47 | | | |
| | MP20-FF | 1.20 ± 0.71 | | | |
| | MP21-FF | 4.36 ± 1.24 | | | |
| | MP22-FF | 3.41 ± 1.95 | | | |
| | MP23-FF | 1.53 ± 0.69 | | | |
| | MP24-FF | 11.20 7.77 | | | |

*Only processed food

***Table 4-2:*** *Unpublished results of total fat and cholesterol contents in RTE items and FF meals*

| *Food Group* | *Sample ID* | *Total Fat Content (mg/100 g sample) ±STD* | *Total Fat Content Reference (mg/100 g sample)* | *Cholesterol (mg/100 g fat) ±STD* | *Cholesterol Reference (mg/100 g fat)* | *Serving Size* |
|---|---|---|---|---|---|---|
| Dairy | D1-RTE | 17.67 ± 2.45 | 18.40 | 20.30 ± 15.85 | 333.33 | 19.00 g |
| | D2-RTE | 26.92 ± 8.47 | 33.00 | 196.33 ± 29.43 | 333.33 | 21.00 g |
| | D3-RTE | 49.71 ± 2.56 | 50.00 | ND | N/A | 1 tbsp |
| | *D4-RTE | 87.62 ± 1.91 | 78.57 | 305.90 ± 41.16 | 272.73 | 14.00 g |
| | D5-RTE | 10.27 ± 0.38 | 10.00 | 165.09 ± 30.33 | 285.71 | 30.00 mL |
| | D6-RTE | 24.16 ± 6.98 | 28.00 | 287.08 ± 20.03 | 300.00 | 28 .00g |
| | D7-RTE | 42.82 ± 1.68 | 35.00 | 238.86 ± 133.50 | 333.33 | 28.00 g |
| | D8-RTE | 12.78 ± 3.04 | 23.40 | 243.02 ± 40.22 | 357.14 | 0.50 cup (65.00 g) |
| | D9-RTE | 1.60 ± 0.31 | 3.03 | 368.25 ± 72.77 | 0.00 | 1 container (99.00g) |
| | D10-RTE | 2.14 ± 0.54 | 0.96 | 282.18 ± 52.16 | 500.00 | 414.03 g |
| | D11-RTE | 12.56 ± 0.38 | 0.69 | 16.25 ± 3.59 | NS | 1 scoop (9.00 g) |
| Meat & Poultry | MP1-RTE | 23.45 ± 5.00 | 20.00 | 224.75 ± 15.73 | 333.33 | 38.00 g |
| | MP2-RTE | 17.84 ± 2.65 | 26.00 | 648.56 ± 125.37 | 250.00 | 32.00 g |
| | MP3-RTE | 0.60 ± 0.071 | 0.65 | 327.69 ± 28.27 | 200.00 | 240.00 mL |
| | MP4-RTE | 3.64 ± 0.018 | 3.30 | 157.80 ± 55.79 | 500.00 | 1 can (236.59 g) |
| | MP5-RTE | 3.53 ± 0.55 | 5.65 | 396.25 ± 46.38 | 500.00 | 11.00 oz (227.00 g) |
| | MP6-RTE | 1.37 ± 0.31 | 1.76 | 2,023.90 ± 209.36 | 750.00 | 0.5 cup (113.40 g) |
| | MP7-RTE | 3.61 ± 0.013 | 0.61 | 352.50 ± 166.24 | 583.33 | 0.5 cup (240.00 g) |
| | MP8-RTE | 2.91 ± 0.53 | 2.87 | 62.55 ± 8.88 | 142.86 | 1 cup (249.00 g) |
| | MP9-RTE | 4.61 ± 0.097 | 4.28 | 195.13 ± 101.17 | 181.82 | 1 cup (257.00 g) |
| | MP10-FF | 33.13 ± 5.12 | 10.00 | 370.80 ± 55.74 | 267.22 | 95.00 g |
| | MP11-FF | 19.52 ± 1.46 | 20.00 | 229.99 ± 53.08 | 222.05 | 4 pieces (64.00 g) |
| | MP12-FF | 23.59 ± 3.40 | 14.00 | 362.03 ± 8.90 | 296.86 | 119.00 g |
| | MP13-FF | 9.83 ± 0.45 | 11.40 | 446.38 ± 73.73 | 464.29 | 1 taco (102.00 g) |

*Table 4-2 (cont'd)*

| *Food Group* | *Sample ID* | *Total Fat Content (mg/100 g sample) ±STD* | *Total Fat Content Reference (mg/100 g sample)* | *Cholesterol (mg/100 g fat) ±STD* | *Cholesterol Reference (mg/100 g fat)* | *Serving Size* |
|---|---|---|---|---|---|---|
| Meat & Poultry | MP14-FF | 11.06 ± 2.09 | 7.60 | 299.73 ± 46.37 | 230.77 | 1 quesadilla (170.00 g) |
| | MP15-FF | 9.95 ± 0.67 | 9.90 | 504.08 ± 7.82 | 245.31 | 1 burrito (140.00 g) |
| | MP16-FF | 12.48 ± 0.30 | 12.21 | 742.86 ± 62.13 | 820.56 | 1 piece (75.00 g) |
| | MP17-FF | 19.39 ± 2.55 | 18.60 | 728.22 ± 42.86 | 600.00 | 1 piece (60.00 g) |
| | MP18-FF | 6.26 ± 1.21 | 4.58 | 333.59 ± 105.66 | 171.43 | 5.40 oz (153. 09 g) |
| | MP19-FF | 8.19 ± 0.78 | 10.80 | 310.55 ± 147.36 | 1,423.08 | 5.70 oz (161.69 g) |
| | MP20-FF | 14.23 ± 2.82 | 10.50 | 526.02 ± 61.70 | 312.92 | 1 sandwich (187.00 g) |
| | MP21-FF | 7.12 ± 1.18 | 6.51 | 450.17 ± 13.91 | 272.73 | 1 sandwich (71.28 g) |
| | MP22-FF | 10.74 ± 4.18 | 8.79 | 335.74 ± 109.38 | 240.74 | 1 sandwich (94.61 g) |
| | MP23-FF | 12.59 ± 1.35 | 11.50 | 280.77 ± 38.10 | N/A | 1 slice (123.00 g) |
| | MP24-FF | 10.96 ± 2.70 | 11.50 | 266.45 ± 30.50 | 200.00 | 1 slice (79.00 g) |
| Seafood | S1-RTE | 3.62 ± 0.25 | 3.38 | 43.89 ± 1.81 | 50.00 | 18.80 oz (532.97 g) |
| | S2-FF | 22.82 ± 1.07 | 14.08 | 216.98 ± 26.53 | 204.39 | 1 sandwich (131.00 g) |
| | S3-FF | 21.70 ± 0.15 | 13.10 | 405.14 ± 37.49 | 555.56 | 5.00 oz (141.75 g) |
| Eggs and derivatives | E1-RTE | 77.68 ± 1.95 | 75.00 | 98.44 ± 16.35 | 90.91 | 1 tbsp |
| | E2-RTE | 14.64 ± 1.02 | 13.91 | 274.11 ± 40.94 | 218.75 | 0.50 cup (115.00 g) |
| Baby food | BF1-RTE | 1.80 ± 0.15 | 1.41 | 1,247.23 ± 227.45 | 1,000.00 | 71.00 g |
| | BF2-RTE | 7.28 ± 0.19 | 4.40 | 673.84 ± 98.50 | 833.33 | 4.00 oz (113.40 g) |
| | BF3-RTE | 2.99 ± 0.06 | 4.40 | 58.17 ± 8.50 | 250.00 | 4.00 oz (113.40 g) |
| | BF4-RTE | 3.25 ± 0.23 | 4.40 | 319.43 ± 83.74 | 500.00 | 4.00 oz (113.40 g) |
| | BF5-RTE | 3.23 ± 0.14 | 4.40 | 348.65 ± 82.26 | 400.00 | 4.00 oz (113.40 g) |
| | BF6-RTE | 2.12 ± 0.60 | 1.56 | 123.24 ± 29.61 | 7.81 | 128.00 g |
| | BF7-RTE | 3.09 ± 0.16 | 4.40 | 263.77 ± 76.69 | 400.00 | 4.00 oz (113.40 g) |
| | BF8-RTE | 6.00 ± 0.14 | 4.90 | 683.55 ± 310.63 | 875.00 | 71.00 g |

*Table 4-2 (cont'd)*

| *Food Group* | *Sample ID* | *Total Fat Content (mg/100 g sample) ±STD* | *Total Fat Content Reference (mg/100 g sample)* | *Cholesterol (mg/100 g fat) ±STD* | *Cholesterol Reference (mg/100 g fat)* | *Serving Size* |
|---|---|---|---|---|---|---|
| Baby Foods | BF9-RTE | 2.79 ± 0.44 | 4.40 | 162.73 ± 95.67 | 333.33 | 4.00 oz (113.40 g) |
| | BF10-RTE | 4.63 ± 0.48 | 4.40 | 251.02 ± 57.95 | 333.33 | 4.00 oz (113.40 g) |
| | BF11-RTE | 3.89 ± 0.23 | 4.40 | 313.34 ± 27.54 | 285.71 | 4.00 oz (113.40 g) |
| | BF12-RTE | 2.90 ± 0.38 | 4.40 | 172.87 ± 17.84 | 333.33 | 4.00 oz (113.40 g) |
| | BF13-RTE | 1.39 ± 0.25 | 2.35 | 327.40 ± 87.35 | 500.00 | 85.00 g |
| Others | O1-RTE | 19.97 ± 1.85 | 26.60 | ND | N/A | 2 tbsp (30.00 g) |
| | O2-RTE | 45.11 ± 6.32 | 50.00 | 51.55 ± 11.01 | 66.67 | 2 tbsp (30.00g) |
| | O3-RTE | 3.65 ± 1.51 | 5.00 | 97.51 ± 32.67 | 333.33 | 2.5 oz (70.90 g) |
| | O4-RTE | 8.70 ± 5.00 | 5.00 | 37.95 ± 8.46 | 285.71 | 1 package (58.00 g) |
| | O5-FF | 13.92 ± 1.58 | 20.00 | ND | 0.00 | 1 medium serving (117.00 g) |
| | O6-FF | 16.27 ± 2.86 | 16.00 | ND | 222.99 | 1 biscuit (76.00 g) |
| | O7-FF | 6.29 ± 1.34 | 6.00 | 268.72 ± 43.02 | 0.00 | 3 hotcakes (149.00 g) |
| | O8-FF | 18.16 ± 0.75 | 17.30 | 16.42 ± 8.31 | 5.87 | 1 biscuit (49.00 g) |
| | O9-FF | 15.96 ± 0.21 | 15.00 | ND | 0.00 | 1 order (34.99 g) |
| | O10-FF | 1.46 ± 0.52 | 3.11 | 90.13 ± 21.47 | 0.00 | 1 order (16.85 g) |

N/A = not apply, ND = not detected, NS = not shown in nutritional label, *Only processed food

*Table 4-3: Unpublished results of COPs concentrations in RTE items*

| | Dairy | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *7α-OH* | *7β-OH* | *4β-OH* | *5,6α-Epoxy* | *5,6β-Epoxy* | *7-Keto* | *Triol* | *6-Keto* | *20α-OH* | *22-OH* | *24-OH* | *25-OH* | *Total COPs* |
| | *(mg/100 g fat) ±STD* | | | | | | | | | | | | |
| *D1-RTE* | 0.15 ± 0.049 | 0.14 ± 0.080 | ND | ND | ND | ND | 0.0 | ND | ND | ND | ND | 1.05 ± 1.21 | 63.59 ± 25.32 |
| *D2-RTE* | 0.084 ± 0.034 | 0.086 ± 0.026 | ND | ND | ND | 0.044 ± 0.029 | 0.051 ± 0.0050 | ND | ND | ND | ND | ND | 0.55 ± 0.13 |
| D3-RTE | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 0.053 ± 0.020 | 0.14 ± 0.048 |
| *D4-RTE | 0.64 ± 0.29 | 0.51 ± 0.21 | ND | 0.044 ± 0.025 | 0.050 ± 0.019 | 0.37 ± 0.13 | 0.18 ± 0.12 | 0.046 ± 0.036 | ND | ND | ND | ND | 2.00 ± 0.92 |
| D5-RTE | 0.19 ± 0.0061 | 0.12 ± 0.029 | ND | ND | ND | 0.11 ± 0.016 | ND | ND | ND | ND | ND | ND | 1.29 ± 0.39 |
| D6-RTE | 0.32 ± 0.10 | 0.27 ± 0.036 | ND | ND | ND | 0.11 ± 0.025 | 0.051 ± 0.0056 | ND | ND | ND | ND | ND | 1.72 ± 0.26 |
| D7-RTE | 0.51 ± 0.26 | 0.39 ± 0.20 | ND | 0.023 ± 0.0046 | 0.058 ± 0.011 | 0.20 ± 0.094 | 0.14 ± 0.032 | 0.046 ± 0.020 | ND | ND | ND | ND | 1.41 ± 0.52 |
| D8-RTE | 0.039 ± 0.019 | 0.043 ± 0.018 | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | 0.21 ± 0.081 |
| D9-RTE | 0.36 ± 0.056 | 0.35 ± 0.072 | ND | ND | ND | 0.16 ± 0.020 | 0.10 ± 0.013 | 0.065 ± 0.038 | ND | ND | ND | ND | 4.06 ± 0.98 |
| *D10-RTE | 2.75 ± 1.88 | 4.72 ± 3.26 | ND | 0.20 ± 0.16 | ND | 1.40 ± 0.92 | 2.49 ± 1.84 | 0.19 ± 0.038 | ND | 0.15 ± 0.068 | ND | 0.43 ± 0.26 | 16.09 ± 8.01 |
| *D11-RTE | 0.075 ± 0.0094 | 0.09 ± 0.033 | ND | ND | ND | ND | ND | ND | 0.055 ± 0.032 | ND | ND | ND | 0.90 ± 0.061 |

*Table 4-3 (cont'd)*

| | 7α-OH | 7β-OH | 4β-OH | 5,6α-Epoxy | 5,6β-Epoxy | 7-Keto | Triol | 6-Keto | 20α-OH | 22-OH | 24-OH | 25-OH | Total COPs |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Meat & Poultry* | | | | | | | | | | | | | |
| | *(mg/100 g fat) ± STD* | | | | | | | | | | | | |
| MP1-RTE | 5.95 ± 5.43 | 4.83 ± 4.30 | ND | 0.10 ± 0.064 | 0.11 0. | 1.93 ± 1.57 | 0.15 ± 0.076 | 0.065 ± 0.029 | ND | 0.068 ± 0.042 | ND | 0.39 ± 0.32 | 15.27 ± 12.80 |
| MP2-RTE | 4.20 ± 1.20 | 3.76 ± 1.06 | ND | 0.12 ± 0.026 | 0.13 ± 0.040 | 1.14 ± 0.41 | 0.058 ± 0.0071 | 0.052 ± 0.020 | ND | ND | ND | 0.36 ± 0.12 | 10.25 ± 2.80 |
| MP3-RTE | 0.081 ± 0.019 | 0.060 ± 0.027 | ND | ND | ND | 0.015 ± 0.0084 | ND | ND | ND | ND | ND | ND | 0.25 ± 0.027 |
| MP4-RTE | 0.96 ± 0.14 | 1.13 ± 0.19 | ND | ND | ND | 0.29 ± 0.021 | 0.22 ± 0.072 | ND | ND | 0.10 ± 0.034 | ND | 0.14 ± 0.095 | 6.99 ± 1.85 |
| MP5-RTE | 0.42 ± 0.11 | 0.32 ± 0.024 | ND | ND | ND | 0.16 ± 0.099 | 0.082 ± 0.012 | 0.079 ± 0.020 | ND | 0.17 ± 0.0081 | 0.050 ± 0.031 | 0.11 ± 0.021 | 2.17 ± 0.22 |
| MP6-RTE | 15.85 ± 3.91 | 16.07 ± 4.20 | ND | 0.26 ± 0.12 | 0.41 ± 0.13 | 3.75 ± 0.68 | 2.33 ± 0.37 | ND | ND | ND | 0.40 ± 0.084 | ND | 43.12 ± 8.98 |
| MP7-RTE | 0.14 ± 0.029 | 0.15 ± 0.060 | ND | ND | ND | 0.066 ± 0.044 | ND | ND | ND | ND | ND | ND | 0.36 ± 0.13 |
| MP8-RTE | 0.54 ± 0.062 | 0.47 ± 0.066 | ND | ND | 0.13 ± 0.022 | 0.12 ± 0.039 | 0.053 ± 0.020 | ND | ND | 0.66 ± 0.043 | ND | 0.19 ± 0.0078 | 3.09 ± 0.16 |
| MP9-RTE | 0.27 ± 0.067 | 0.20 ± 0.070 | ND | ND | ND | 0.085 ± 0.025 | ND | ND | ND | ND | ND | ND | 1.36 ± 0.38 |
| *Seafood* | | | | | | | | | | | | | |
| | 7α-OH | 7β-OH | 4β-OH | 5,6α-Epoxy | 5,6β-Epoxy | 7-Keto | Triol | 6-Keto | 20α-OH | 22-OH | 24-OH | 25-OH | Total COPs |
| | *(mg/100 g fat) ± STD* | | | | | | | | | | | | |
| S1-RTE | 0.052 ± 0.020 | 0.060 ± 0.013 | ND | ND | ND | ND | ND | ND | ND | 0.090 ± 0.013 | ND | ND | 0.25 ± 0.026 |

*Table 4-3* *(cont'd)*

| *Eggs & egg's derivatives* | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *7α-OH* | *7β-OH* | *4β-OH* | *5,6α-Epoxy* | *5,6β-Epoxy* | *7-Keto* | *Triol* | *6-Keto* | *20α-OH* | *22-OH* | *24-OH* | *25-OH* | *Total COPs* |
| | *(mg/100 g fat) ±STD* | | | | | | | | | | | | |
| **E1-RTE** | 0.11 ± 0.051 | 0.13 ± 0.024 | ND | ND | ND | 0.062 ± 0.021 | ND | ND | 0.025 ± 0.017 | 0.069 ± 0.077 | ND | 0.22 ± 0.036 | 0.91 ± 0.15 |
| **E2-RTE** | 0.47 ± 0.27 | 0.32 ± 0.19 | ND | ND | ND | 0.18 ± 0.088 | ND | ND | ND | ND | ND | ND | 1.35 ± 0.60 |
| *Baby Food* | | | | | | | | | | | | | |
| | *7α-OH* | *7β-OH* | *4β-OH* | *5,6α-Epoxy* | *5,6β-Epoxy* | *7-Keto* | *Triol* | *6-Keto* | *20α-OH* | *22-OH* | *24-OH* | *25-OH* | *Total COPs* |
| | *(mg/100 g fat) ±STD* | | | | | | | | | | | | |
| ***BF1-RTE*** | 8.97 ± 5.42 | 24.61 ± 15.21 | ND | 0.69 ± 0.36 | 1.39 ± 0.81 | 16.69 ± 5.45 | 2.63 ± 0.46 | ND | ND | ND | ND | 3.60 ± 1.22 | 62.55 ± 25.55 |
| ***BF2-RTE*** | 2.92 ± 0.73 | 2.27 ± 0.64 | ND | 0.044 ± 0.012 | 0.033 ± 0.0062 | 0.84 ± 0.34 | 0.22 ± 0.031 | 0.11 ± 0.064 | ND | ND | ND | 0.24 ± 0.055 | 8.48 ± 2.46 |
| **BF3-RTE** | 0.10 ± 0.031 | 0.065 ± 0.037 | ND | ND | ND | ND | ND | ND | 0.65 ± 0.17 | 0.33 ± 0.11 | 0.18 ± 0.47 | ND | 2.65 ± 0.46 |
| **BF4-RTE** | 1.80 ± 0.22 | 1.61 ± 0.24 | ND | ND | ND | 0.57 ± 0.065 | 0.27 ± 0.086 | ND | ND | 0.20 ± 0.044 | ND | 0.16 ± 0.056 | 7.32 ± 1.08 |
| **BF5-RTE** | 2.10 ± 0.50 | 1.94 ± 0.37 | ND | 0.052 ± 0.021 | 0.045 ± 0.027 | 0.59 ± 0.18 | 0.31 ± 0.091 | ND | ND | 0.10 ± 0.034 | ND | 0.27 ± 0.16 | 6.44 ± 1.23 |
| **BF6-RTE** | 3.73 ± 1.98 | 0.66 ± 0.12 | ND | ND | ND | 0.67 ± 0.42 | 0.20 ± 0.034 | ND | ND | 0.077 ± 0.031 | ND | ND | 8.04 ± 3.16 |
| **BF7-RTE** | 2.12 ± 0.16 | 2.38 ± 0.15 | ND | ND | ND | 0.48 ± 0.15 | 0.39 ± 0.056 | ND | ND | 0.17 ± 0.069 | ND | ND | 6.13 ± 0.30 |
| **BF8-RTE** | 5.85 ± 1.82 | 4.91 ± 1.12 | ND | 0.030 ± 0.019 | 0.073 ± 0.039 | 1.68 ± 0.61 | 0.27 ± 0.10 | 0.12 ± 0.018 | ND | ND | ND | 0.67 ± 0.16 | 14.02 ± 3.97 |
| **BF9-RTE** | 0.68 ± 0.12 | 0.59 ± 0.17 | ND | ND | ND | 0.24 ± 0.075 | 0.21 ± 0.050 | ND | ND | ND | ND | ND | 8.14 ± 5.70 |

*Table 4-3 (cont'd)*

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***BF10-RTE*** | 2.08 ± 0.63 | 1.62 ± 0.46 | ND | 0.027 ± 0.016 | 0.042 ± 0.032 | 0.65 ± 0.087 | 0.35 ± 0.041 | ND | ND | 0.067 ± 0.015 | ND | 0.27 ± 0.047 | 6.36 ± 1.36 |
| ***BF11-RTE*** | 0.66 ± 0.092 | 0.58 ± 0.020 | ND | ND | 1.05 ± 0.12 | ND | 0.12 ± 0.036 | ND | ND | 2.48 ± 1.45 | ND | ND | 5.28 ± 1.49 |
| ***BF12-RTE*** | 0.41 ± 0.10 | 0.40 ± 0.078 | ND | ND | ND | 0.18 ± 0.013 | 0.10 ± 0.044 | ND | ND | 0.18 ± 0.11 | ND | ND | 2.05 ± 0.31 |
| ***BF13-RTE*** | 4.22 ± 3.77 | 1.27 ± 0.89 | ND | 0.062 ± 0.023 | 0.10 ± 0.025 | 1.31 ± 0.83 | 0.15 ± 0.03 | ND | ND | 0.12 ± 0.017 | 0.14 ± 0.029 | 0.47 ± 0.32 | 11.58 ± 7.07 |
| ***Others*** | | | | | | | | | | | | | |
| | ***7α-OH*** | ***7β-OH*** | ***4β-OH*** | ***5,6α-Epoxy*** | ***5,6β-Epoxy*** | ***7-Keto*** | ***Triol*** | ***6-Keto*** | ***20α-OH*** | ***22-OH*** | ***24-OH*** | ***25-OH*** | ***Total COPs*** |
| | ***(mg/100 g fat) ± STD*** | | | | | | | | | | | | |
| ***O1-RTE*** | 0.12 ± 0.018 | 0.094 ± 0.023 | ND | ND | ND | ND | 0.053 ± 0.014 | 0.023 ± 0.011 | ND | 0.099 ± 0.027 | ND | ND | 1.56 ± 0.28 |
| ***O2-RTE*** | 0.11 ± 0.037 | 0.089 ± 0.015 | ND | ND | ND | 0.035 ± 0.0079 | 0.060 ± 0.016 | ND | 0.031 ± 0.020 | ND | ND | ND | 0.61 ± 0.12 |
| ***O3-RTE*** | 0.57 ± 0.11 | 0.60 ± 0.14 | ND | 0.10 ± 0.030 | 0.055 ± 0.043 | 0.20 ± 0.060 | 0.13 ± 0.055 | ND | ND | 0.083 ± 0.0022 | ND | ND | 5.60 ± 1.28 |
| ***O4-RTE*** | 1.19 ± 0.78 | 1.22 ± 0.79 | ND | 0.066 ± 0.028 | 0.054 ± 0.053 | 0.30 ± 0.095 | 0.17 ± 0.063 | 0.078 ± 0.020 | ND | 0.051 ± 0.027 | ND | 0.15 ± 0.10 | 9.58 ± 2.73 |

*Only processed food

*Table 4-4: Unpublished results of COPs concentrations in FF meals*

| Meat & Poultry | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *7α-OH* | *7β-OH* | *4β-OH* | *5,6α-E* | *5,6β-E* | *7-Keto* | *Triol* | *6-Keto* | *20α-OH* | *22-OH* | *24-OH* | *25-OH* | *Total COPs* |
| | *(mg/100 g fat) ± STD* | | | | | | | | | | | | |
| *MP10-FF* | 3.06 ± 1.37 | 2.28 ± 1.21 | ND | 0.051 ± 0.0052 | 0.068 ± 0.024 | 0.92 ± 0.48 | 0.12 ± 0.057 | ND | ND | ND | 0.14 ± 0.094 | 0.21 ± 0.12 | 8.64 ± 3.04 |
| *MP11-FF* | 0.31 ± 0.031 | 0.29 ± 0.048 | ND | ND | ND | 0.12 ± 0.052 | 0.033 ± 0.015 | 0.045 ± 0.034 | 0.11 ± 0.060 | 0.21 ± 0.17 | ND | 0.17 ± 0.013 | 1.91 ± 0.084 |
| MP12-FF | 1.00 ± 0.29 | 0.72 ± 0.28 | ND | ND | ND | 0.35 ± 0.083 | 0.030 ± 0.020 | 0.050 ± 0.0052 | ND | ND | 0.10 ± 0.024 | 0.11 ± 0.044 | 3.18 ± 0.65 |
| MP13-FF | 0.26 ± 0.023 | 0.28 ± 0.041 | ND | ND | ND | 0.077 ± 0.16 | 0.024 ± 0.012 | ND | ND | ND | ND | 0.12 ± 0.036 | 1.67 ± 0.20 |
| MP14-FF | 0.75 ± 0.044 | 0.55 ± 0.086 | ND | 0.056 ± 0.038 | 0.13 ± 0.041 | 0.54 ± 0.21 | 0.21 ± 0.037 | 0.070 ± 0.021 | 0.13 ± 0.036 | 0.20 ± 0.030 | ND | 0.2 2 ± 0.038 | 14.64 ± 11.61 |
| MP15-FF | 1.89 ± 0.66 | 0.78 ± 0.10 | 0.49 ± 0.030 | 0.061 ± 0.013 | 0.11 ± 0.016 | 0.86 ± 0.33 | 0.12 ± 0.040 | ND | ND | ND | ND | ND | 5.02 ± 1.08 |
| MP16-FF | 0.69 ± 0.20 | 0.75 ± 0.22 | ND | 0.056 ± 0.037 | 0.044 ± 0.023 | 0.20 ± 0.033 | 0.053 ± 0.017 | ND | 0.050 ± 0.017 | ND | ND | ND | 3.90 ± 1.00 |
| MP17-FF | 0.19 ± 0.44 | 0.19 ± 0.037 | ND | ND | ND | 0.63 ± 0.018 | 0.031 ± 0.018 | ND | ND | ND | ND | ND | 1.55 ± 0.38 |
| MP18-FF | 3.04 ± 0.32 | 0.99 ± 0.42 | ND | 0.023 ± 0.011 | 0.029 ± 0.0099 | 0.57 ± 0.089 | 0.067 ± 0.023 | 0.039 ± 0.014 | ND | ND | ND | ND | 5.72 ± 0.99 |
| MP19-FF | 0.48 ± 0.044 | 0.34 ± 0.036 | ND | 0.030 ± 0.020 | 0.028 ± 0.0057 | 0.20 ± 0.040 | 0.11 ± 0.026 | 0.021 ± 0.0069 | ND | 0.10 ± 0.032 | ND | 0.049 ± 0.029 | 1.75 ± 0.077 |
| MP20-FF | 0.18 ± 0.060 | 0.16 ± 0.040 | ND | ND | ND | 0.057 ± 0.035 | 0.037 ± 0.031 | ND | ND | ND | ND | 0.090 ± 0.055 | 0.91 ± 0.11 |
| MP21-FF | 4.01 ± 0.081 | 3.50 ± 0.14 | ND | 0.060 ± 0.019 | 0.12 ± 0.041 | 1.42 ± 0.24 | 0.11 ± 0.038 | 0.069 ± 0.052 | 0.019 ± 0.0073 | 0.019 ± 0.016 | 0.18 ± 0.029 | 0.12 ± 0.009 9 | 10.40 ± 0.71 |
| MP22-FF | 1.81 ± 0.23 | 2.61 0.29 | ND | ND | ND | 0.71 ± 0.034 | 0.071 ± 0.019 | ND | ND | ND | ND | ND | 5.40 ± 0.56 |

*Table 4-4 (cont'd)*

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **MP23-FF** | 16.80 ± 9.61 | 10.23 ± 4.14 | 0.41 ± 0.22 | 0.21 ± 0.078 | 0.52 ± 0.28 | ND | 0.60 ± 0.32 | 0.22 ± 0.081 | 0.16 ± 0.045 | 0.43 ± 0.11 | ND | 1.29 ± 0.64 | 39.13 ± 18.94 |
| **MP24-FF** | 0.14 ± 0.036 | 0.18 ± 0.035 | ND | ND | ND | ND | 0.036 ± 0.012 | ND | ND | ND | ND | 0.15 ± 0.064 | 2.53 ± 0.33 |
| | | | | | | *Seafood* | | | | | | | |
| | *7α-OH* | *7β-OH* | *4β-OH* | *5,6α-Epoxy* | *5,6β-Epoxy* | *7-Keto* | *Triol* | *6-Keto* | *20α-OH* | *22-OH* | *24-OH* | *25-OH* | *Total COPs* |
| | | | | | | *(mg/100 g fat) ± STD* | | | | | | | |
| **S2-FF** | 0.33 ± 0.075 | 0.23 ± 0.029 | ND | ND | ND | 0.15 ± 0.012 | 0.092 ± 0.029 | ND | ND | ND | ND | 0.16 ± 0.079 | 2.66 ± 0.44 |
| **S3-FF** | 1.37 ± 0.83 | 1.27 ± 0.72 | ND | 0.055 ± 0.040 | 0.18 ± 0.037 | 0.43 ± 0.25 | 0.12 ± 0.069 | 0.081 ± 0.034 | 0.12 ± 0.065 | 0.20 ± 0.025 | ND | 0.17 ± 0.042 | 7.27 ± 2.85 |
| | | | | | | *Others* | | | | | | | |
| | *7α-OH* | *7β-OH* | *4β-OH* | *5,6α-Epoxy* | *5,6β-Epoxy* | *7-Keto* | *Triol* | *6-Keto* | *20α-OH* | *22-OH* | *24-OH* | *25-OH* | *Total COPs* |
| | | | | | | *(mg/100 g fat) ± STD* | | | | | | | |
| **05-FF** | 0.096 ± 0.036 | 0.093 ± 0.033 | 0.031 ± 0.0090 | 0.021 ± 0.0041 | ND | 0.084 ± 0.028 | ND | ND | ND | ND | 0.15 ± 0.039 | ND | 3.16 ± 1.11 |
| **06-FF** | 0.52 ± 0.31 | 0.30 ± 0.13 | ND | ND | ND | 0.26 ± 0.18 | 0.10 ± 0.017 | ND | 0.16 ± 0.10 | 0.18 ± 0.017 | ND | tr | 1.97 ± 0.61 |
| **07-FF** | 0.81 ± 0.038 | 0.60 ± 0.044 | ND | 0.068 ± 0.036 | 0.080 ± 0.018 | 0.22 ± 0.021 | 0.075 ± 0.015 | ND | 0.11 ± 0.042 | ND | ND | ND | 2.40 ± 0.14 |
| **08-FF** | 0.11 ± 0.073 | 0.096 ± 0.045 | ND | ND | ND | ND | 0.021 ± 0.013 | ND | ND | 0.024 ± 0.019 | ND | 0.16 ± 0.033 | 3.14 ± 1.56 |
| **09-FF** | 0.099 ± 0.037 | 0.082 ± 0.033 | ND | ND | ND | 0.056 ± 0.027 | 0.033 ± 0.0065 | ND | 0.052 ± 0.013 | ND | ND | 0.19 ± 0.049 | 9.62 ± 2.24 |
| **010-FF** | 0.42 ± 0.052 | 0.37 ± 0.060 | ND | ND | 0.10 ± 0.022 | ND | 0.041 ± 0.017 | ND | ND | 0.52 ± 0.030 | ND | 0.15 ± 0.011 | 2.33 ± 0.21 |

## 4.6 DISCUSSION

### 4.6.1 Fat in UPFs

From the 19 RTE items that showed higher amounts of fat compared to their nutritional fact label or the FDA database, 10 are either dairy products (Figure 3-1) or products that contain a dairy product as an ingredient (D4-RTE, S1-RTE, D5-RTE, D7-RTE, O4-RTE, MP7-RTE, D10-RTE, D11-RTE, MP8-RTE, MP9-RTE). All these RTE items are prepared with milk, which could contain up to 3.25% of fat (whole milk), in addition to other ingredients with high fat content such as oils.

Another 5 of the RTE items with a high fat content are baby foods: BF2-RTE, BF8-RTE, BF6-RTE, and BF10-RTE. Some of the ingredients of BF10-RTE are: canola oil and ground chicken, which could contribute to its high fat content. However, this means that children are also exposed to develop different chronic disease, such as obesity (Ogden et al., 2014), due to the unmonitored consumption of these foods manufactured for infants and children which contains such high levels of fat. In fact, the US Centers for Disease Control and Prevention growth charts state that “nearly one-quarter of 2-to 5-year-old children in the United States have overweight or obesity defined as at or above the 85th and 95$^{th}$ percentiles, respectively, for age- and sex-adjusted body mass index (BMI)”. (Emond et al., 2020)

Three additional RTE items with high fat content contained meat (specifically, beef) as their principal ingredient. Beef is a meat high in fat content that can be observed from roasted rib roast large end which contains 24 g per serving size (3 oz) (USDA, 2011), to ground beef which can contain a maximum 30% fat allowed by the USDA (USDA, 2016). E1-RTE was the last RTE item with a high fat content. Mayonnaise contains egg yolk which is the

part of the egg with a higher fat content. One small egg could have 5 g per serving size (1 egg = 50g). (ENC, n.d.)

From the FF meals, the obtained fat content was drastically different from the value obtained from the USDA food database or each FF's webpage. Seventeen out of 23 FF meals showed a fat content higher than the one mentioned in either the USDA food database or the FF's nutritional information found on their webpages. FF meals are known to be served in large portions, containing high levels of saturated fat and added sugars. (Harris et al., 2013; Rosenheck, 2008) Most of these FF meals are prepared with several different ingredients such as oils, eggs, high-fat meats; all of them containing high fat content themselves, resulting in a meal with an overall high fat content.

RTE and FF meals are the main contributors of fat accumulation in the body due to their high fat levels. (Mohiuddin & Nasirullah, 2020) Fat accumulation in the body is directly related to different chronic diseases such as obesity, diabetes, hypertension, atherosclerosis, among others (Shori et al., 2017). Therefore, the quantification of total fat in RTE items and FF meals is of great importance to corroborate the actual fat intake of adults and children who consume UP foods in the USA.

### 4.6.2 Secondary Oxidation Products (TBARS)

TBARS assay has been widely used as a validation technique for lipid oxidation in food (Miller et al., 1994; Permal et al., 2020; Zeb & Ullah, 2016; Y. Zhang et al., 2019). The determination of secondary oxidation products by MDA quantification confirms the extent of lipid oxidation in a food sample. No differences were observed between FF and RTE samples but were observed between food groups ($p < 0.001$). However, since this test was mainly developed for comparison between solely raw and cooked meat samples (not fat samples of

entire meals with a mix of different ingredients as it was in our case for FF meals and RTE items), using an entire piece of the food matrix, MDA was not able to be quantified in some of our samples. Our hypothesis is that after a certain amount of time and due to all the temperature changes, that these specific meals undergo before, during, and after their final cooking step at home, all the unstable MDA molecules tend to convert in other molecules such as aldehydes and ketones, hindering its measurement during the TBARS assay. In general, MDA could not be detected in more FF meals than RTE items. Eight RTE items were not tested for TBARS.

### 4.6.3 Total Cholesterol

FF was the category with the highest cholesterol content ($p < 0.01$). Overall, cholesterol values were different between their reference values stablished in their nutritional fact label or the FF meals webpages. Sixteen out of 23 FF meals showed differences up to 179.78% in cholesterol content. From these 16 FF meals with high cholesterol content, 2 of these meals (010-FF and 07-FF) showed cholesterol quantities of 90.13 and 268.72 mg/100 g fat, respectively, even though their values from the data base and FF's webpages reported to be 0 mg/100g fat.

09-FF and 05-FF showed 0 mg/100g fat of cholesterol which was confirmed by the USDA's food data base and each of the FF's webpages. These items are mainly made of wheat flour which contains no cholesterol. MP23-FF had a total cholesterol content of 504.08 mg/100g fat; however, its value could not be confirmed by any source since this item has been discontinued from the market.

As it was mentioned above, FF meals also showed for most of their items, a higher fat content compared to the values obtained from the USDA food database, or each individual

FF webpage or Eat this much and Fast-Food Nutrition webpages. A positive relationship can be seen with fat content and cholesterol content, which could help us to further understand COPs formation in foods.

The food group with the highest average cholesterol content was meat and poultry, reaching a value of 440.69 mg/100 g fat. The 2nd group was baby foods with cholesterol contents up to 380.40 mg/100 g fat (Figure 4-4).

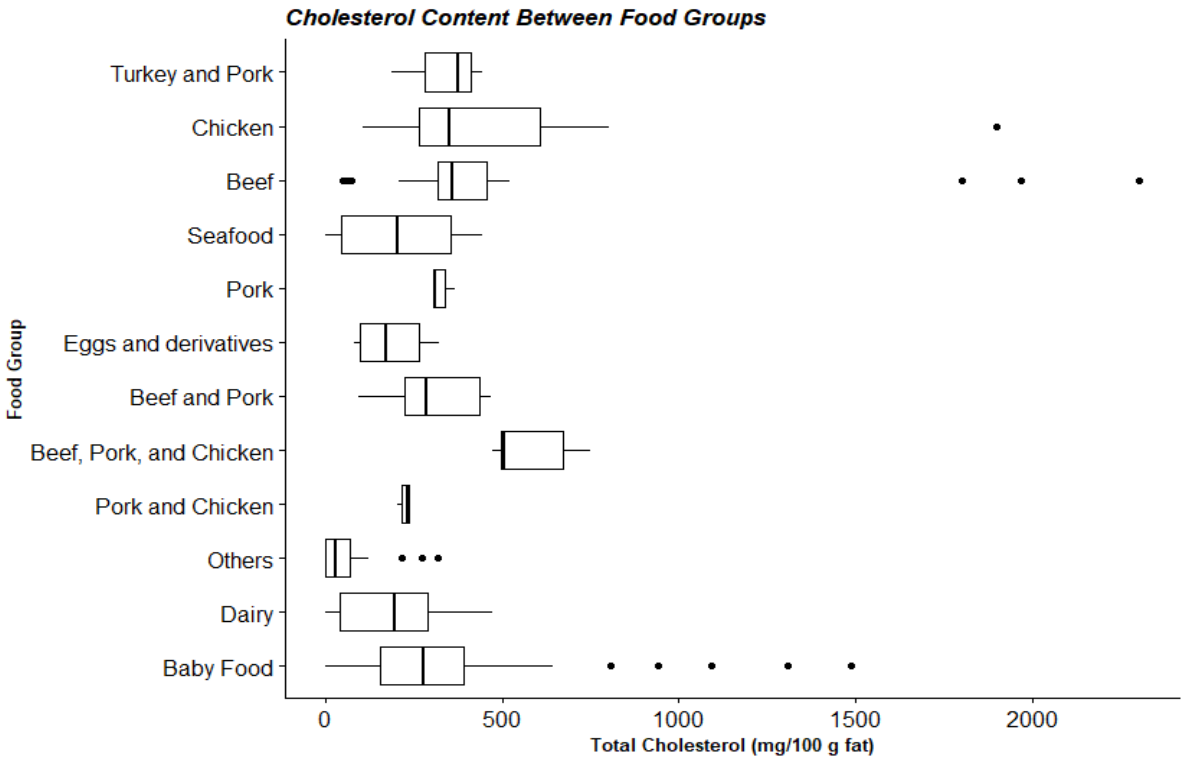


***Figure 4-4:*** *Distribution of cholesterol between food groups*

### 4.6.4 Phytosterols and Tocopherols in UPFs

The food matrix and ingredients added to the food item throughout its preparation plays an important role in this phytosterols distribution for being compounds mostly derived from

oils and vegetable origin ingredients. Canola oil, olive oil, avocado, and other vegetables are some examples of ingredients that are added to different RTE items and FF meals to enhance the product's taste and make it suitable for the consumer's palate.

Overall, FF contained higher amounts of phytosterols. Food groups with the highest phytosterols content were: pork, eggs and egg's derivatives, baby foods, and "others". The RTE item with the highest content was MP3-RTE.

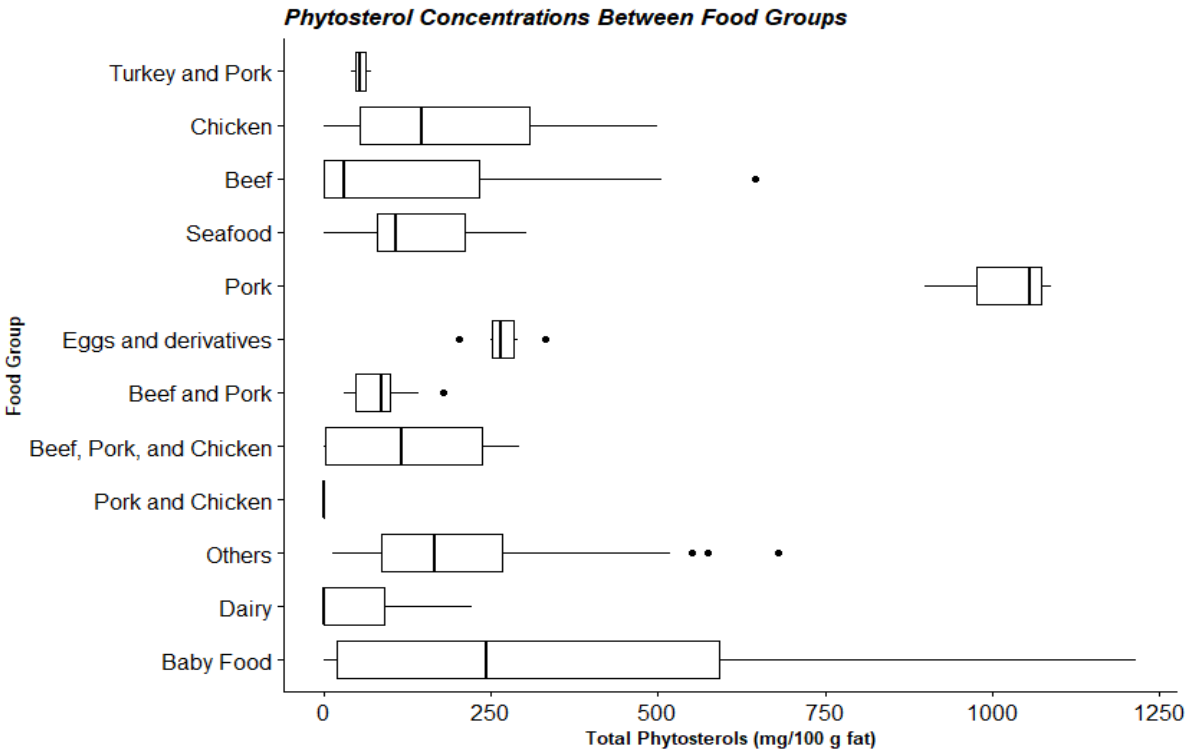


***Figure 4-5:*** *Distribution of phytosterols between food groups*

These items, especially baby foods, contain high amounts of canola and other vegetable derivatives which are rich in phytosterols, hence, it was expected to find several phytosterol in these samples; specifically, β-Sitosterol. Sixteen of the RTE items did not presented any phytosterol content which are items were meat, cheese and other animal-origin ingredients

are more dominant. Unlike FF meals' results, RTE items showed presence of Fucosterol and β-Sitosterol.

A total of 21 FF meals showed a total phytosterol amount of ≥ 36.45 ± 17.21 mg/100g fat. 09-FF and 05-FF were the 2 FF meals with the highest total phytosterols content (561.67 ± 83.62 and 523.88 ± 55.47 mg/100g fat, respectively), followed by MP11-FF with 419.52 ± 56.86 mg/100g fat. French fries and chicken nuggets are cooked in the industry using vegetable oil; hence, it was expected to find several phytosterol in these samples; specifically, β-Sitosterol. Just 2 FF meals did not show any phytosterol content (MP10-FF and MP12-FF) mainly because of its dominant meat composition which in turns results in a high cholesterol content as it was previously described. No FF meal showed presence of Fucosterol or β-Tocopherol.

The presence of phytosterols in FF meals and RTE is of great importance since these compounds are also known to be susceptible to different processing condition such as temperature changes, light exposure, and other factors that could result in the formation of other type oxidation products, similar to COPs. (Kilvington et al., 2019; Maldonado-Pereira et al., 2018)

### 4.6.5 COPs Occurrence in the Western Diet

UPFs foods are produced under different and variable conditions. Most of the time, these food products undergo several processing steps as part of the desired final product. Hence, formation of COPs is known to be promoted as an effect of these constant parameters variability during UP manufacturing. (Guardiola et al., 2002; Maldonado-Pereira et al., 2018) Therefore, the database created in this chapter is vital to know their occurrence in the

Western diet. This database will help to understand how FF meals and RTE items' processing techniques affect and promote their formation.

Differences in food composition and processing conditions can be observed in both FF meals and RTE items. In FF meals, total COPs content reached values up to 39.13 mg/100g fat (MP23-FF). On the contrary, the lowest amount was 0.91 mg/100g fat (MP20-FF). For RTE items, the highest value for total COPs was 63.59 mg/100 g fat for D1-RTE, followed by BF1-RTE with 62.55 mg/100 g fat. The lowest COPs contents were: 0.14 mg/ 100 g fat for D3-RTE.

A total of 12 individual COPs were found in our samples: 7α-OH, 7β-OH, 4β-OH 5,6α-Epoxy, 5,6β-Epoxy, Triol, 7-Keto, 6-Keto, 20α-OH, 22-OH, 24-OH, and 25-OH. The food group with the highest amounts of COPs was meat and poultry (Figure 4-5).

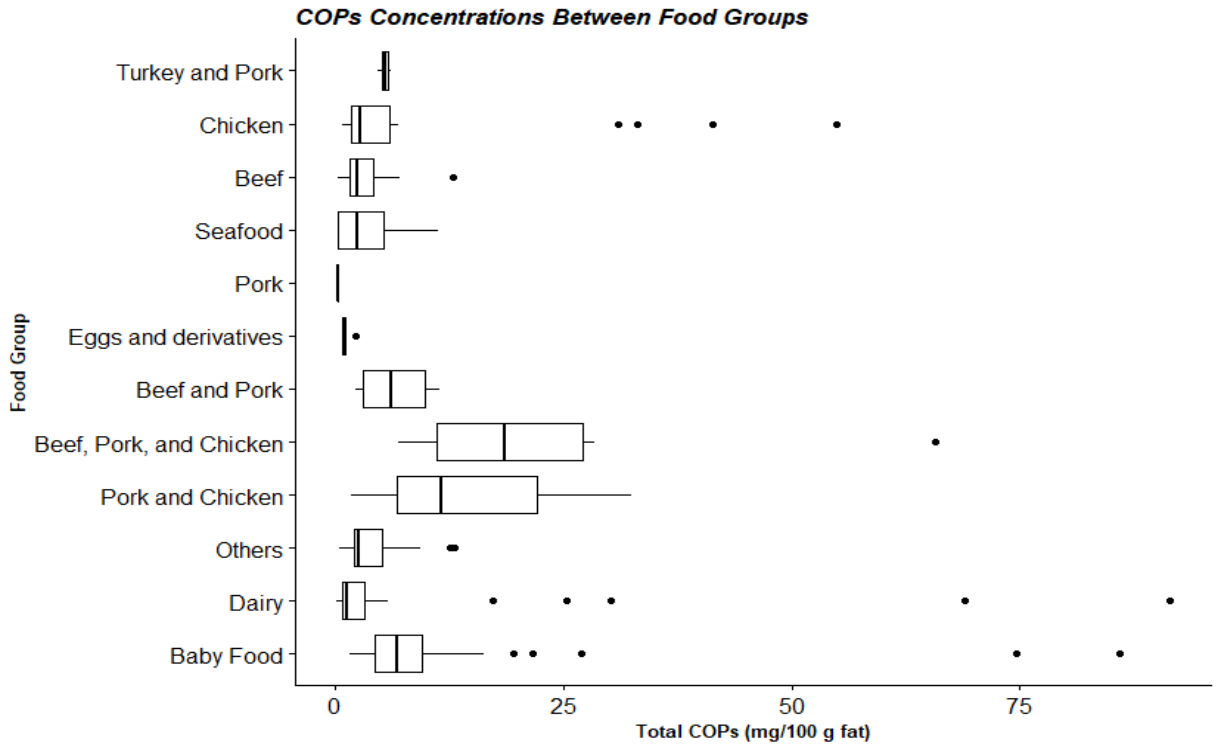


***Figure 4-6:*** *Distribution of COPs between food groups*

Moreover, MP15-FF contained all COPs resulted from the oxidation of cholesterol at carbon-position 7 (C-7). These are: 7α-OH, 7β-OH, triol, 7-Keto. As it was previously mentioned, a relationship with COPs formation and food type is significant ($p < 0.001$) and could help us to better understand COPs formation pathway in all these food items. These thermal changes vary among the food matrix, and the cooking methods and conditions. Hence, COPs formation, which are the predominant group within the lipid oxidation products, becomes not only directly related to the food matrix composition, but also to the processing method, packing and storage conditions, which are standardized and completely different from one franchise or brand to another. For RTE items, a total of 11 COPs were found in our samples: 7α-OH, 7β-OH, 5,6α-Epoxy, 5,6β-Epoxy, Triol, 7-Keto, 6-ketocholestanol, 20α-OH, 22-OH, 24-OH, and 25-OH. No presence of 4β-OH was found. This difference marks a distinction between RTE items and FF meals. However, since this is the first study performed to investigate FF meals and RTE items and there are not many studies focused on the potential relationship between COPs, and FF meals and RTE items, there is not much information that we could use to compare our results and deeply evaluate all these changes observed after our analyses. For general items such as egg products, French fries and beef hamburger, some studies can be found ((Bonoli et al., 2008; Broncano et al., 2009; Brzeska et al., 2016; Hur et al., 2007; Maldonado-Pereira et al., 2018; Medina-Meza, Rodriguez-Estrada, et al., 2014; Medina-Meza & Barnaba, 2013; Nielsen et al., 1996; Paniangvait et al., 1995; Won Park & Addis, 1987; Zardetto et al., 2014; W. B. Zhang et al., n.d.) and used for general comparison.

It is known that the presence of 7-Keto in oxidized samples marks the completion of the lipid oxidation mechanism, being 7-Keto one of the last COPs formed based on reaction

rates previously studied (I. Medina-Meza & C. Barnaba, 2013). For most of our samples, 7-Keto was found in considerable quantities. At this point, a kinetics analysis to validate these reaction rate values is required to obtain this critical information regarding COPs formation and development in FF meals and RTE items.

## 4.7 CONCLUSIONS

Fat was significantly higher ($p < 0.001$) in FF meals compared to RTE items. Also, fat was positively correlated to the amount of SFAs ($p < 0.001$), while PUFAs are positively correlated with phytosterols content ($p < 0.001$), which at the same time, were directly related with the use of vegetables oils and other ingredient such a fish, avocado, among others, which contain high PUFA levels. High amounts of fat tend to result in a high amount of cholesterol for those FF meals and RTE items dominated by animal-origin ingredients. However, several items that had a reference cholesterol value of 0 mg/100 g fat, showed actual values other than 0, meaning there is some incongruency between the information provided to the consumer and each item's actual value. MDA values of RTE items were, in general, higher than those of FF meals. However, the inability of detecting MDA in FF meal could mean that the lipid oxidation extent was higher in the FF meals, because of the possible presence of other secondary oxidation products formed after the decomposition of MDA molecules such as aldehydes, ketones, among others. Lastly, cholesterol was positively related with COPs, but some variability between food matrixes and cooking parameters was observed. The UPF with the highest COPs amount was D1-RTE. However, the food group with the highest total COPs value was the baby food group.,

7$\alpha$-OH and 7$\beta$-OH were present in all FF meals, except for D3-RTE. All baby foods showed presence of 7$\alpha$-OH, but phytosterols basically dominated this group of RTE items.

# CHAPTER 5 : LIPID OXIDATION SPECIES IN THE WESTERN DIET – A HEALTH AND NUTRITION THREAT

## 5.1 OVERALL SIGNIFICANCE

This study directly impacts four main areas: Food safety, Nutritional value, Food Market, and Public Health described in detail in the following sections and Figure 5.1. This project enhances the current nutritional knowledge of oxidized lipid on food and food products provided to the consumers through the nutritional label, by generating information of the presence of COPs as well as their relationship with chronic diseases. It is expected that the decision-making process of laws and regulations ensuring food safety through the implementation of a standardized and correct procedure of processing techniques will be improved, considering these potentially hazardous compounds.

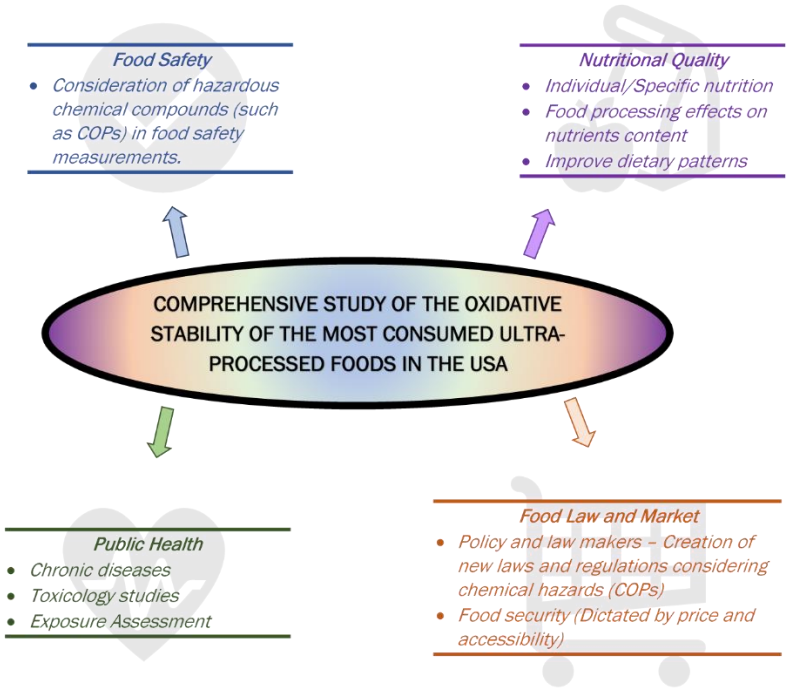


***Figure 5-1:*** *Study's significance in the three main areas: Public Health, Nutrition, and Food Market*

## 5.2 LIPID OXIDATION AND FOOD SAFETY

For decades, food safety has been focused on avoiding food borne illnesses and microbial reduction (FoodSafety.gov, 2020a). Therefore, different processing conditions and methods have been developed following the suggestions and conditions required to reduce the risk of getting any food-sick or microbial infection. Unfortunately, the food market has never considered other potentially harmful compounds that could result from current processing techniques in the food industry. They focus on complying with the regulations stablished by the government without knowing the existence of other hazardous species. This is the case of COPs and other lipid oxidation species that have been overlooked for the past 30 years despite their association with different chronic diseases (Bang et al., 2008; S. Lemaire et al., 1998; Lemaire-Ewing et al., 2005; Maldonado-Pereira et al., 2018; Snelson et al., 2021). Chapter 1 through 4 were developed to prove the need of a more "chemical" point of view of food safety by their adverse health effects which have been reported recently in several studies (Joshi, Darshane, & Beke, 2020; Snelson et al., 2021). These chapters suggest a deeper analysis of the unpredictable formation of these lipid oxidation species in food because of the variability in food processing technologies and cooking conditions to ensure a chemically safe food product.

## 5.3 LIPID OXIDATION AND NUTRITIONAL VALUE

The effect of processing conditions in the food quality was demonstrated in Chapter 3. Chapter 3 highlights the nutritional changes in fatty acids composition resulting from the variability of parameters as part of manufacturing processes used in the food industry. This chapter proved that these conditions, which are developed to ensure the manufacturing of a safe and secure food product, play a crucial role in the overall nutritional content of foods. A

deeper analysis of the nutritive changes and the unpredictable formation of these lipid oxidation species in food because of the variability in food processing methods and conditions was suggested to ensure a nutritious product. This project enhanced the current nutritional knowledge of food and food products provided to the consumers through the nutritional label, by generating information of the presence of COPs and other antioxidant molecules. Dietitians and nutritionist will benefit from this study by obtaining a detailed chemical and nutritional report of the most consumed 63 ultra-processed foods in the US which will help them to better understand and acknowledge the presence of these potential hazardous species in the US diet. It is expected that this information will start to be considered in the evaluation of healthy diet options for the US population.

## 5.4 LIPID OXIDATION AND THE FOOD LAW

Food law governs food production, distribution, and consumption to protect the consumers against unsafe, adulterated, and misbranded food (Kotwal, 2016). It is also an important area impacted by this study. The most consumed UPFs by the US population are RTE and FF, which account for 60% of the calorie intake per day (C. a. C. G. a. L. M. a. L. M. L. a. M. P. Monteiro, 2019) and are considered one of the most cheap and available food options. Therefore, the food industry and policy makers will benefit from this novel information about the COPs content in UPFs according to different food technologies. New information regarding the processing and manufacturing of their products will help to start correcting or modifying any processing step(s) categorized as “harmful” for human health. Policy makers will obtain new and critical nutritional, health, and even economical knowledge regarding food safety. This study observed an inverse trend between prices and calories per serving which emphasizes the effect of product affordability and food security

with dietary patterns. Laws and regulations can be modified considering new health threats such as the adverse effects of these lipid oxidation species such as COPs. In general, the food industry, including law and policy makers will acquire information that will improve the decision-making process of new food laws and regulations to ensure the well-being of us citizens.

## 5.5 LIPID OXIDATION SPECIES AND HUMAN HEALTH

The significance of this study relies mainly on COPs and other lipid oxidation species such as POPs (specifically, their effects related to infant formula consumed by infants during their first months of life) as a new Public Health issue. Chapters 1 and 2 remarked the adverse effects on human health of COPs and highlighted the need of a database of these compounds in the Western diet, which was addressed in Chapter 4. This project increased the current knowledge of the occurrence, and prevalence, of COPs in the Western diet, by the creation of a complete database of COPs present in the Western diet available to be used in new toxicology studies, by food scientists, food engineers, nutritionists, dietitians, as well as the general public. The new information derived from this project, will provide a baseline for future nutritional, toxicological, and epidemiological studies in COPs that will expand current medical knowledge of chronic diseases related to these compounds. Novel nutritional quality information of food products, the presence of COPs and their relationship with chronic diseases can be available to consumers, with the possibility to change/modify their eating patterns to healthier alternatives. The use of this database could create a paradigm shift regarding the use of calorie content as major parameter when designing diets, allowing for more individual and specific nutritional and medical treatments. Additionally, Chapter 4 suggested an Absorption, Distribution, Metabolism and Excretion

(ADME) study as the next specific step to address COPs as a potential health threat. Results from the ADME study will enable the performance of more detailed exposure assessments, and eventually, risk assessments.

### 5.5.1 Dietary Exposure Assessments as a Public Health Tool

An exposure model is an evaluating method of the intake of a compound of concern over a specified period. It describes variability and uncertainty across populations and sub-populations considering different socio-economics parameters such as age, gender, education level, annual income, race, ethnicity, etc. It is also part of the risk assessment that determines the exposure dose to be combined with a "dose – response model" to estimate risk. Exposure can be expressed as a function of magnitude, duration, and frequency. The significance of performing an exposure assessment is described in (Figure 5.2).

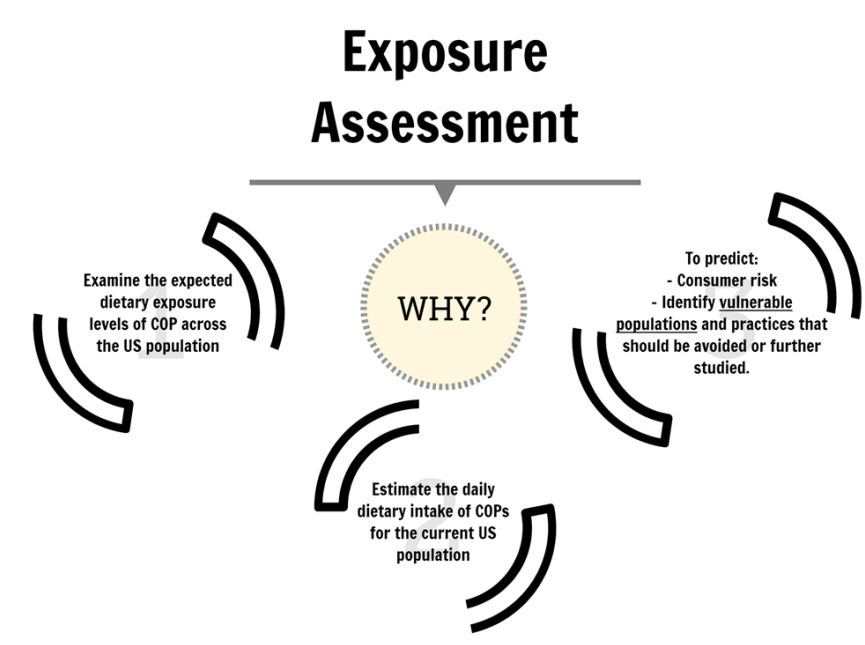


***Figure 5-2:*** *Importance of an Exposure Assessment*

### 5.5.2 SHEDS-HT Exposure Model

The Stochastic Human Exposure and Dose Simulation (SHEDS) was the exposure model modified and tested using COPs values obtained from this study. SHEDS was developed by the US Environmental Protection Agency (EPA) and has been used to create different versions of predictive models for a variety of chemicals. It can characterize in detail the variability and uncertainty in population exposures using demographic, exposure factor, and chemical application data, in combination with human activity and location information. SHEDS has been successfully applied for consumer product ingredients and agricultural pesticides such as organophosphate and pyrethroid pesticides (Buck, Ozkaynak, Xue, Zartarian, & Hammerstrom, 2001; Hore et al., 2006; Tulve et al., 2011; V. Zartarian et al., 2012; V. G. Zartarian et al., 2000), arsenic (J. Xue et al., 2006; J. P. Xue, Zartarian, Wang, Liu, & Georgopoulos, 2010; V. Zartarian et al., 2006), and methyl mercury (J. P. Xue, Zartarian, Liu, & Geller, 2012). However, it has not been utilized yet for any dietary exposure assessment.

SHEDS-High Throughput (SHEDS-HT) was developed as a leaner and more versatile version that included the **direct ingestion pathway** and allows it to be applied quickly to a large number of chemicals. SHEDS-HT was chosen because of its **benefits and characteristics**:

- It is run in R (mostly available for free)
- It has potential to generate population distributions of daily level exposures and intake doses (mg/kg-body weight/day) for a range of chemicals present in residential environments, foods, and drinking water in a HT capacity.

- It models active chemical exposure pathways for a given compound for the simulated population
- Calculates aggregate chemical exposures for each individual
- Uses Monte Carlo methods to assign relevant exposure factors and cohort-matched activity and food intake diaries to each person
- Ingestion exposures are based on a fraction of mass that is ingested during the consumption of the food item.

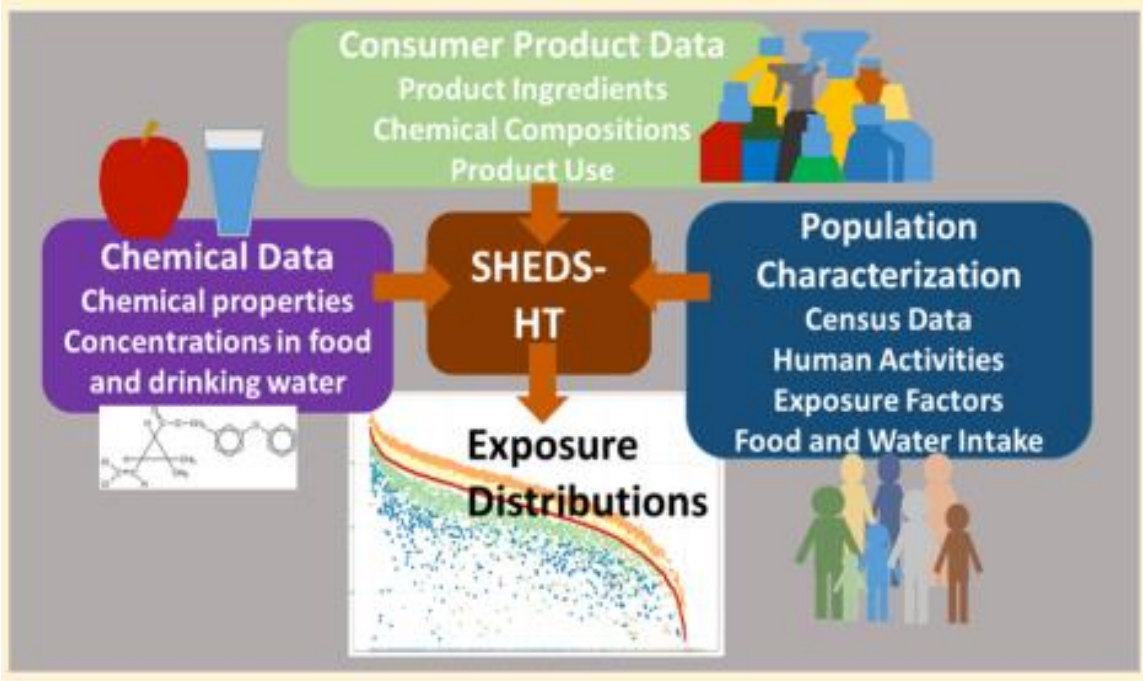


***Figure 5-3:*** *SHEDS Input Categories (Isaacs et al., 2014)*

Figure 5.3 shows the three main input categories that are required to run SHEDS-HT: ***(1) Population Characterization***: US Census, and Dietary diaries from NHANES-WWEIA: 1999-2006, ***(2) Chemical Data***: chemical and physical properties of tested COPs, and their

concentration in the foods tested in this study; *(3) Consumer Product Data*: Since this is a Dietary Exposure Assessment, this category was not needed for our exposure assessment.

#### 5.5.2.1 How Does SHEDS Work?

The model includes 4 main files that were optimized to include the UPFs, COPs concentrations and their chemical and physical properties needed to enable their analysis:

1. chem_props.txt = contains all the chemical and physical properties of the compounds analyzed.
2. diet_diaries.txt = contains daily intakes in [g/day] for various foods. Each record corresponds to one person for one day. The default diary file contains aggregated summaries of food consumption data from the NHANES-What We Eat In America Study (1999-2006, USDA 2014; NHANES-WWEIA). Food consumption in grams is given for each of the SHEDS-HT default foods, for each person in the study. The variables on the file are the age, gender, body weight in [kg], and the intake quantities. The food groups are indicated by short labels (usually two characters). These are matched in SHEDS with distributions from the source.chemical input file. Food "XX" on the diet.diaries file is matched to the source name beginning with "FOOD; XX:" on the source.chemicals file. The user may use their own choice of food groups provided both input files are consistent.
3. source_chem_food.txt = contains distributions for modeling variables that depend on both the source and the specific chemical. The recognized distributional forms are Bernoulli, binomial, beta, discrete, empirical, exponential, gamma, lognormal, normal, point, probabilistic, and triangle. These divide into two categories. The ones that return a set of discrete values are Bernoulli, binomial, discrete, empirical, point, and probabilistic.

The continuous distributions are beta, exponential, gamma, lognormal, normal, point, and triangle. A “point” may be used when either type is expected. In this study, concentration distributions for each food were obtained from Chapter 4.

4. source_scen_food.txt. = chemicals sources to be included in the run and the scenarios to be run for each. SHEDS currently has nine scenarios (see Table 1), each of which has a column on the source_Scen_food file. The first column contains the source type: one of food, product, or article. The second column is the source.ID, which is a brief label used for matching data to other input files. Each line on this file must have a unique source.ID, but otherwise they are arbitrary, and the user may add new ones. The third column is a description of the source. This is not used by the model (the source.ID is always used internally). The fourth column is a flag indicating whether the source in considered to be indoors (1) or outdoors (0), which may impact the inhalation. There is one column for each exposure scenario. The source type must match the first part of the scenario name. But not all appropriate scenarios need to be active. A “1” indicates that the scenarios are active, or “0” means that the combination of source and scenario will not be evaluated.

Dietary exposures in SHEDS-HT are calculated by determining the total daily mass of the compound intake for each simulated person via different foods (Eqn. 5.1). For each simulated individual, daily COP concentrations (μg of total COPs per g of food) are sampled (one for each food item). Dietary exposures are calculated as the sum (over all UPFs listed in the database) of the product of concentration and mass of food consumed (assigned food diary for the person).

$$\boldsymbol{COP_x\ intake} = \frac{Food\ intake\left(\frac{kg}{day}\right)}{individual^{'}\ sweight\ (kg)} \times\ COP_x\ conc.\ in\ Food\left(\frac{mg}{kg\ of\ Food - day}\right) \quad \text{(Eqn. 5.1)}$$

$$Total\ COPs\ intake = \sum COP_i = COP_x + COPs_y + COP_z \ldots \quad \text{(Eqn. 5.2)}$$

$$\boxed{COP_x\ intake = \left(\frac{mg\ COP_x}{kg - day}\right)} \quad \text{(Eqn. 5.3)}$$

#### 5.5.2.2 Population Module

A population of many thousands of individuals can be handled by the model. Input data was based on the U.S. Census and was used to generate a simulated population representative of the U.S. population.

#### 5.5.2.3 Exposure Assessment Test – 2nd Stage Baby Foods

COP values of 12 baby foods (from BF1-RTE to BF12-RTE) were obtained from the study results showed in Chapter 4 (Table 4-3). Additional information regarding baby food samples such as brand, flavors, and serving sizes can be found in APPENDIX C.

Daily intake values for each baby food were obtained from the What We Eat in America (WWEIA) food commodity database ((EPA), 2018). Chemical properties such as water solubility, molecular weight, and absorption factor for each COP analyzed were obtained from PubChem.com (NIH, 2021) and the Human Metabolome Database (TMIC, 2021). RStudio (version 1.4.1106 © 2009-2021) was used to run SHEDS-HT.

Results showed that infants (6-12 months) could be exposed to 0.0031 mg/kg/6 mo. (Table 5-1) It is worth mentioning that our results could be underestimating the exposure

level reported in Table 5-1 since the intake rate of infants (6-12 months old) for various baby food combination meals obtained from the WWEIA database was not available. No specific dietary value was available for each individual baby food type and mixture meals. Therefore, average values of single-ingredient baby foods were used to represent baby food meals' main ingredients.

***Table 5-1:*** *Unpublished results of COPs exposure in infant (6-12 months old)*

| *Total COPs Exposure using SHEDS-HT* | | | |
|---|---|---|---|
| 50% Percentile | | 95% Percentile | |
| *Exposure 3 months (mg/kg/day)* | *Exposure 6 months (mg/kg/day)* | *Exposure 3 months (mg/kg/day)* | *Exposure 6 months (mg/kg/day)* |
| 0.000047633 | 0.000095265 | 0.001527069 | 0.003054138 |

## 5.6 CONCLUSIONS

Lipid oxidation species (such as COPs and POPs) are a new health and nutrition issue that requires urgent action. These compounds are promoted by different processing conditions currently employed in the food industry. Their association with several chronic diseases and their adverse biological effects that negatively impacts human health was covered in Chapters 1 and 2. These chapters pointed the importance of considering these compounds as part of food safety processes. Additionally, it marked the need of more studies that can clarify the lack of knowledge of the occurrence of these compounds, specifically, in the Western diet.

UPFs nutritional quality, specially, unavoidable changes in macronutrients quantities resulting from the heterogeneity of the manufacturing processes, were covered in Chapter 3. This chapter can provide to the reader with nutritional and non-nutritional information regarding the effects of concurrent consumption of UPFs and, hopefully, will help the food

industry, policy makers, and US population to modify dietary recommendations leaning towards a healthier and chemically safe diet. It is critical to obtain the support of law makers that will ensure the creation of new regulations that will considerate these lipid oxidation species as hazardous for human health.

Information provided in Chapter 4 is a valuable tool for future studies that will elucidate the current knowledge of these species and their exact mechanism in the human body. An ADME study is essential for next research steps and it is scheduled as part of this dissertation author's future working plans together with a complete and more robust exposure assessment.

This study has a direct impact in the areas of public health, food safety, nutrition quality, and food law and market (Chapter 5). Moreover, results from an exposure assessment test showed that an infant could be ingesting a minimum of 2.86 ug per serving by consuming just 12 baby food items studied in this project. The purpose of the addition of ingredients to baby foods, which are the source of these sterols, is to fulfill nutritional infants' needs. However, these oxidative compounds have not been fully analyzed in infants from a toxicological point of view. SHEDS-HT model shows that an infant could be ingesting 0.0031 mg/kg/6 mo. Since there is no study addressing the effects of COPs on infants' health, no assumption could be done to determine if it should be a health risk. Once again, an ADME study and a risk assessment are imperative to understand the effect of these toxic compounds on infants.

## 5.7 FUTURE WORK

An ADME study was established in Chapter 4 as the next step to address COPs as a potential health threat. Critical values/rates of absorption, deposition, metabolism, and

excretion of individual COPs can now be determined together with the COPs values obtained in this project. This information is vital to expand the current knowledge of COPs on metabolism (including impacts on the microbiome). In addition, once the absorbance values are obtained from the toxicological analysis, they can be used to perform an exposure assessment that will provide insight of the level of exposure of COPs in the US population, and eventually, a risk assessment.

# APPENDICES

# APPENDIX A

## THE ROLE OF CHOLESTEROL OXIDATION PRODUCTS IN FOOD TOXICITY (JOURNAL PAPER)

*Figure 6: The Role of Cholesterol Oxidation Products in Food Toxicity (Journal Paper)*

Food and Chemical Toxicology 118 (2018) 908–939

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Review

# The role of cholesterol oxidation products in food toxicity

Lisaura Maldonado-Pereira[a], Matthew Schweiss[b], Carlo Barnaba[c,**], Ilce Gabriela Medina-Meza[a,b,*]

[a] Department of Chemical Engineering and Materials Science, Michigan State University, East Lansing, MI, USA
[b] Department of Biosystems and Agricultural Engineering, Michigan State University, East Lansing, MI, USA
[c] Biophysics Program and Department of Chemistry, The University of Michigan, Ann Arbor, MI, USA

ARTICLE INFO

*Keywords:*
Cholesterol oxidation products
Risk assessment
Exposure
Food toxicity
Food biomarkers

ABSTRACT

Food consumption can lead to the accumulation of certain chemical compounds able to exert toxic activities against humans. Of mayor interests are those molecules generated during food processing and handling, since their occurrence and distribution depend of many intrinsic and extrinsic factors. Cholesterol – a lipid constituent of mammalian cells – is the precursor of several toxic molecules known as cholesterol oxidation products (COPs). In the last decades, it has been demonstrated that food processing can dramatically trigger COPs accumulation in meats, eggs, dairy products, fish and poultry. On the other hand, countless scientific evidences have pointed out the highly toxic and pathogenic activities of COPs, from cancer stimulation to neurodegenerative disorders, *via* molecular mechanisms that are largely unexplored. The aim of this review is to merge the evidence on COPs accumulation in foods and their toxic activities through dietary intake, as from *in vivo* and *in vitro* studies. We consider that it is imperative to systematically monitor the formation of COPs to bridge these quantitative efforts with a risk exposure assessment on sensitive populations.

## 1. Introduction

Cholesterol is a key component of mammalian cells, with undoubtedly biological significance. It is a precursor of a variety of hormones, vitamin D and bile acid production cycle (Olkkonen et al., 2017). Cholesterol is synthetized endogenously in the body in amounts necessary to sustain life. The presence of reactive oxygen species (ROS) due to cell oxidative stress or exposure to radical species and precursors increases cholesterol susceptibility to oxidation (Medina-Meza and Barnaba, 2013; Smith, 1996). This non-enzymatic oxidation occurs at the C5-C6 double bond *via* nucleophilic attack by radicals and other reactive species. Cholesterol side-chain is also susceptible of radical attack (Iuliano, 2011; Smith, 1996). ROS-mediated oxidation can be triggered by light (Maerker and Jones, 1993; Medina-Meza et al., 2012; Medina-Meza et al., 2014a,b), heat (Chien et al., 1998; Derewiaka and Molinska nee Sosinska, 2015), radiation (Maerker and Jones, 1993), metal ions (Hur et al., 2007b; Jo et al., 1999) and other agents that have the ability to lower the energetic requirements for the reaction to occur (Beltran et al., 2004; Medina-Meza and Barnaba, 2013). Cholesterol oxidation produces a group of molecules that preserve the steroidal motif of the parent compound, but with an additional hydroxyl, ketone, or epoxy group (Savage et al., 2002) (Fig. 1). They are commonly known as cholesterol oxidation products (COPs), and depending on the site of oxidation, COPs can be distinguished as A-ring, B-ring or side-chain oxidation products (Table 1 and Fig. 1), each with demonstrated biochemical, biological and even biophysical activities in mammalian cells and subcellular components (Leonarduzzi et al., 2005). Endogenous COPs have been considered as key intermediates in bile acid and steroid hormone biosynthesis or as autoxidation products in tissues, especially under conditions of oxidative stress (Vaughn et al., 1997; Olkkonen et al., 2012; Ikegami et al., 2014). Exogenous COPs, on the other hand, are mainly derived from diet by consumption of high cholesterol-containing foods. This intake results in an accumulation of COPs in several organs and tissues, as extensively reported (Biasi et al., 2013; Miyoshi et al., 2014; Ogino et al., 2007; Ros, 2000). Among foods, animal food products including meat and meat products (Al-Ismail, 2002; Al-Ismail et al., 2007; Bilska et al., 2009; Britt et al., 1998; Chen et al.; Grau et al., 2001; Novelli et al., 1998; Osada et al., 2000; Zubillaga and Maerker, 1991; Hur et al., 2015), fish (Echarte et al., 2004; Hernandez Becerra et al., 2014; Osada et al., 1993; Zunin et al., 2001), eggs and egg products (Chen et al., 2010), cheese (Nourooz-Zadeh and Appelqvist, 1988), milk (Calderón-Santiago et al., 2012; Dionisi et al., 1998) and other dairy products (Angulo et al., 1997; Gorassini et al., 2017; Kumar

* Corresponding author. Michigan State University, 469 Wilson Rd., Room 302C, East Lansing, MI 48824, USA.
** Corresponding author. University of Michigan, Ann Arbor, MI, 48109, USA.
*E-mail addresses:* carlob@umich.edu (C. Barnaba), ilce@msu.edu (I.G. Medina-Meza).

https://doi.org/10.1016/j.fct.2018.05.059
Received 15 March 2018; Received in revised form 17 May 2018; Accepted 25 May 2018
Available online 27 June 2018
0278-6915/ Published by Elsevier Ltd.

*Figure 6:* *(cont'd)*



**List of abbreviations:**

| | |
|---|---|
| COPs | cholesterol oxidation products |
| CHOL | cholesterol |
| ROS | reactive oxygen species |
| 7-OH | 7-hydroxycholesterol |
| 7-keto | 7-ketocholesterol |
| 5,6α-epoxy | 5α,6α-epoxycholesterol |
| 5,6β-epoxy | 5β,6β-epoxycholesterol |
| LDL | low density lipoprotein |
| HDL | high density lipoprotein |
| VLDL | very low-density lipoprotein |
| LXR | liver X receptor |
| 7α-OH | 7α-hydroxycholesterol |
| 7β-OH | 7β-hydroxycholesterol |
| 24S-OH | 24S-hydroxycholesterol |
| 27-OH | 27-hydroxycholesterol |
| AR | androgen receptor |
| MS | multiple sclerosis |
| 25-OH | 25-hydroxycholesterol |
| 20-OH | 20α-hydroxycholesterol |
| Triol | Cholestane-3β,5α,6β-triol |
| HD | Huntington's disease |
| AD | Alzheimer's disease |
| MCI | mild cognitive impairment |
| PUFA | polyunsaturated fatty acid |
| DHA | docosahexaenoic acid |
| ARA | arachidonic acid |
| RTE | ready to eat |
| TDS | total dietary study |
| FDA | food and drug administration |
| ACAT | acyl-coenzyme A cholesterol acetyltransferase |
| UHT | ultra-high temperature |
| U937 | Human lymphocyte cells |
| MCF7 | Human mammary gland, breast; derived from metastatic site: pleural effusion |
| CYP | cytochrome P450 enzyme family |
| HUVEC | Human umbilical vein endothelial cell |

and Singhal, 1992; Mariutti and Bragagnolo, 2017; Nielsen et al., 1995; Sun and Holley, 2010) are the major source of dietary COPs (Grandgirard et al., 2002; Ogino et al., 2007; Hur et al., 2007a). The content of COPs in food sources has been largely overlooked, despite a consistent body of evidences on their biological and pathological activities in humans. COPs are unavoidable and unintentionally formed during food processing, storage, handling and even household preparations, making human exposure a tangible risk.

In this review, we will address the gap between the scientific evidence of COPs formation in biological systems, and the chemical exposure and toxicological significance through dietary intake. First, we will discuss data regarding the occurrence of COPs in foods and other biological matrices. Second, the compound-specific biological and pathological significance will be addressed. Then, we will discuss the factors that enhance their formation in food products. We will finally highlight the importance of estimate the fate of these compounds through a risk exposure assessment. Overall, this review will strengthen the need of a holistic approach on cholesterol oxidation and COPs, an emerging field already known as "cholesterolomics", which will be helpful in the assessment of novel chemical biomarkers for food safety and toxicology.

## 2. COPs occurrence in biological systems

### 2.1. Absorption and transport of COPs

The fate of dietary cholesterol and cholesterol-derivatives has been studied in different animal models, including humans. Dietary

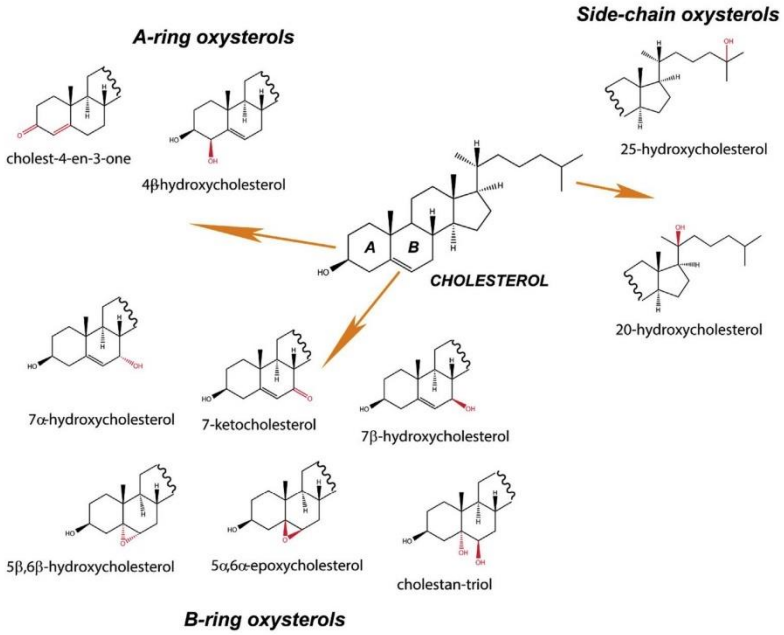


**Fig. 1.** Structure of the main COPs classified according to the site of oxidation.

*Figure 6:* *(cont'd)*



**Table 1**
Endogenous and exogenous origin of the most detected COPs.

| Common name | Abbreviation | Location* | | | Formation Pathway | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | *Endogenous origin* | | | *Exogenous origin* |
| | | Side-chain | Ring A | Ring B | Enzymatic formation/ Enzyme** | | Non-enzymatic formation (ROS) | Autoxidation /Dietary Intake |
| 27-hydroxycholesterol | 27-OH | X | | | X | 27-hydrolase (CYP27A1) | | |
| 24S-hydroxycholesterol | 24S-OH | X | | | X | 24-hydrolase (CYP46A1) | | |
| 22-hydroxycholesterol | 22-OH | X | | | X | 22-hydrolase (CYP11A1) | | |
| 25-hydroxycholesterol | 25-OH | X | | | X | 25-hydrolase (Ch25h), CYP3A4 | X | X |
| 20α-hydroxycholesterol | 20-OH | X | | | | | | X |
| 4β-hydroxycholesterol | 4β-OH | | X | | X | CYP3A4 | | X |
| 7α-hydroxycholesterol | 7α-OH | | | X | X | 7α-hydrolase (CYP7A1) | X | X |
| 7β-hydroxycholesterol | 7β-OH | | | X | | | X | X |
| 7-ketocholesterol | 7-keto | | | X | | | X | X |
| Cholesterol-5α,6α-epoxide | 5,6α-epoxy | | | X | | | X | X |
| Cholesterol-5β,6β-epoxide | 5,6β-epoxy | | | X | | | X | X |
| Cholesterol-3β, 5α, 6β-triol | Triol | | | X | | | X | X |
| 5,6-secosterol | secosterol | | | X | | | | X |
| 3β,5α-dihydroxycholest-7-en-6-one | DHCEO | | | X | X | 7-dehydrocholesterol reductase (DHCR7) | | |

*(Tai et al., 1999) (Mast et al., 2011; Xu et al., 2012).
**(Aldini et al., 2010) (Tai et al., 1999).

cholesterol appears in the lumen of the intestine associated with triglycerides and phospholipids in lipid emulsion. These lipid micelles are digested by lipases, and cholesterol is released for its transport to the brush border of the small intestine for absorption by the mucosal cells (Rozner and Garti, 2006). Cholesterol is absorbed in the human small intestine through ABCG5/ABCG8 and NPC1L1 transporters, which efluxes a portion of absorbed cholesterol back into the small intestinal lumen and drives the cholesterol uptake in the enterocytes (Rozner and Garti, 2006). On the contrary, the absorption pathway of COPs has not been characterized in its completeness due to their chemical diversity, polarity and abundance in the diet. Studies have shown that COPs are absorbed in the small intestine at a lower rate than cholesterol (−66%) (Osada et al., 2014), probably due to their lower solubility in micelles. Cholesterol, fatty acids and eventually COPs are then assembled into large intestinal lipoproteins, called chylomicrons. Cholesterol is esterified by the acyl-coenzyme A cholesterol acetyltransferase (ACAT) before incorporation into the chylomicrons. Intragastric administration of a mixture of COPs in rats revealed that their absorption is related with changes in the composition of lymph chylomicron after 2–4 h (Emanuel et al., 1991). The authors also found a time-correlation between the incorporation of individual COPs species into the lymph chylomicrons. 7-OH isomers were early incorporated (3 h), followed by 7-keto and 5,6α-epoxy at 4 and 5 h, respectively (Vine et al., 1997, 1998). 7-OH isomers and 7-keto are the COPs commonly identified in lipoproteins (Kuver, 2012). The highest levels of COPs are present in low-density lipoproteins (LDLs) and to a lesser extent in high-density lipoproteins (HDLs) and very-low-density lipoproteins (VLDLs) (Vaya et al., 2001; Vejux and Lizard, 2009). Huang et al. (2015) suggested the revision of the cholesterol transport hypothesis, since several epidemiological studies demonstrated the cholesterol efflux (reverse cholesterol transport) play an important role in the absorption and removal of excess of cholesterol in plasma. Notwithstanding, no information is still provided about the implication of COPs in the absorption of cholesterol.

### 2.2. Biological and pathological activities of COPs

In the last decades, there has been mounting evidence that COPs exert biological and pathological activities in both *in vitro* and *in* vivo systems, with potential health concerns for humans (Leonarduzzi et al., 2005). Several studies have demonstrated that COPs can exert pro-inflammatory (Biasi et al., 2013; Lemaire-Ewing et al., 2005; Miyoshi et al., 2014; Virginio et al., 2015), pro-oxidant (Biasi et al., 2009; Mariutti and Bragagnolo, 2017; Seet et al., 2009), pro-fibrogenic (Gargiulo et al., 2016) and pro-apoptotic (Colles et al., 2001; Vejux and Lizard, 2009) activities in several cell lines (Raza et al., 2016; Vejux and Lizard, 2009; Zarrouk et al., 2014). COPs have also shown specific deleterious properties such as cytotoxicity, mutagenicity (Sevanian and Peterson, 1986), carcinogenicity (Homma et al., 2004), Alzheimer's disease (Marwarha and Ghribi, 2015), Parkinson's disease (Bjorkhem et al., 2013; Lee et al., 2009; Leoni and Caccia, 2011), age-onset macular degeneration (Javitt and Javitt, 2009) as well as cataracts (Girao et al., 1998), osteoporosis (Liu et al., 2005), colon carcinoma (Biasi et al., 2009; Roussi et al., 2005), prostate cancer (Fukuchi et al., 2004; Homma et al., 2004; Kulig et al., 2016), and breast cancer (Cruz et al., 2010; Gruenke et al., 1987; Lappano et al., 2011; Wu et al., 2013; Nelson et al., 2014). The potential relationship between COPs and several chronic diseases is summarized in Table 2, accompanied by a brief description of the speculated mechanism(s). Due to the demonstrated effects of COPs in diffused and emerging human pathologies, there is an imperative demand on more accurate and systematic information about COPs intake in targeted populations (i.e. infants, elder people, etc.). Forty five percent of Americans have suffered from some type of chronic illness (2014), and according to the Agency for Healthcare Research and Quality (AHRQ), chronic diseases (including those COPs related diseases), are the most prevalent healthcare issues in the United States (AA.VV, 2015; NIH, 2017). These conditions include arthritis, asthma, cancer, cardiovascular (heart) disease, depression and diabetes, though these are only a few of many chronic illnesses that negatively affect the lives of Americans (AA.VV, 2015). The World Health Organization's Department of Evidence and Research shows that

*Figure 6: (cont'd)*



**Table 2**
COPs toxicity and its role in the major chronic diseases and pathologies. Dietary COPs are marked in light yellow.

| CARDIOVASCULAR DISEASES | | | | |
|---|---|---|---|---|
| *ATHEROSCLEROSIS* | | | | |
| **Oxysterol** | **Affected Tissue** | **Concentration exerting biological activity** | **Effect** | **Reference** |
| **27-OH** | Peripheral artery | 9.27 (29.8) ng/mg tissue | – Oxidative stress (mixture)<br>– Inflammation (alone)<br>– Endothelial dysfunction/cell phenotype changes<br>– Act as agonists of liver X receptors (LXRs). | (Virginio et al., 2015) |
| | Carotid | 432 ± 323 ng/mg tissue | | (Leonarduzzi et al., 2007) |
| | | 250 ± 110 ng/mg tissue | | (Vaya et al., 2001) |
| | Aorta | 640 (80) pmol/mg protein | | (Upston et al., 2002) |
| | Carotid/aorta | 220 ± 225 ng/mg tissue | | (Garcia-Cruset et al., 2001) |
| | Coronary | 250 ± 100 ng/mg tissue | | (Vaya et al., 2001) |
| **24S-OH** | ND | ND | – Apoptosis (alone)<br>– Act as agonists of liver X receptors (LXRs). | (Alkazemi et al., 2008), (Gargiulo et al., 2015) |
| **22-OH** | ND | ND | – Vascular calcification (alone/mixture)<br>– Act as agonists of liver X receptors (LXRs). | (Alkazemi et al., 2008), (Gargiulo et al., 2015) |
| **25-OH** | Peripheral artery | 0.50 (0.64) ng/mg tissue | – Oxidative stress<br>– Inflammation (alone/mixture)<br>– Apoptosis (alone)<br>– Endothelial dysfunction/cell phenotype changes<br>– Vascular calcification<br>– Act as agonists of liver X receptors (LXRs). | (Alkazemi et al., 2008), (Virginio et al., 2015) |
| **7β-OH** | Carotid | 128 (75.6-216.8) ng/mg free cholesterol | – Oxidative stress<br>– Inflammation (alone)<br>– Apoptosis (alone) | (Helmschrodt et al., 2013) |
| | | 3.50 (1.17-20.64) ng/mL | | (Helmschrodt et al., 2013) |
| | | 146 ± 88 ng/mg tissue | – Endothelial dysfunction/cell phenotype changes (alone) | (Leonarduzzi et al., 2007) |
| | | 9.8 ± 7.8 ng/mg tissue | | (Micheletta et al., 2004) |
| | | 7.75 ± 3.9 ng/mg tissue | | (Iuliano et al., 2003) |
| | | 420 ± 724 ng/mg tissue | | (Carpenter et al., 2003) |
| | | 20 ± 10 ng/mg tissue | | (Vaya et al., 2001) |
| | Carotid/aorta | 57 ± 40 ng/mg tissue | | (Garcia-Cruset et al., 2001) |
| | Coronary | 140 ± 90 ng/mg tissue | | (Vaya et al., 2001) |
| **7α-OH** | Carotid | 128 (75.6-216.8) ng/mg free cholesterol | – Oxidative stress<br>– Inflammation<br>– Apoptosis<br>– Endothelial dysfunction/cell phenotype changes<br>– Act as agonists of liver X receptors (LXRs). | (Helmschrodt et al., 2013) |
| | | 3.50 (1.17-20.64) ng/mL | | (Helmschrodt et al., 2013) |
| | Carotid/aorta | 130 ± 110 ng/mg tissue | | (Garcia-Cruset et al., 2001) (Alkazemi et al., 2008) |
| | Coronary | 40 ± 10 ng/mg tissue | | (Vaya et al., 2001) |
| **7-keto** | Carotid | 607.1 (205.9-1407.6) ng/mg free cholesterol | – Oxidative stress<br>– Inflammation<br>– Apoptosis<br>– Endothelial dysfunction/cell phenotype changes<br>– Vascular calcification | (Helmschrodt et al., 2013) |
| | | 6.19 (2.63-30.47) ng/mL | | (Helmschrodt et al., 2013) |
| | | 322 ± 279 ng/mg tissue | | (Leonarduzzi et al., 2007) |
| | | 39.7 ± 14 ng/mg tissue | | (Micheletta et al., 2004) |

(*continued on next page*)

*Figure 6: (cont'd)*



**Table 2** (*continued*)

| | | | | |
|---|---|---|---|---|
| | | 35.9 ± 21.5 ng/mg tissue | | (Iuliano et al., 2003) |
| | | 70 ± 30 ng/mg tissue | | (Vaya et al., 2001) |
| | | 90 (20) ng/mg tissue | | (Upston et al., 2002) |
| | Carotid/aorta | 100 ± 63 ng/mg tissue | | (Garcia-Cruset et al., 2001) |
| | Coronary | 80 ± 40 ng/mg tissue | | (Vaya et al., 2001) |
| **5,6α-epoxy** | Carotid | 110 (34.4-165.4) ng/mg free cholesterol | – Oxidative stress<br>– Inflammation (alone)<br>– Apoptosis (alone)<br>– Endothelial dysfunction/cell phenotype changes (alone) | (Helmschrodt et al., 2013) |
| | | 1.34 (0.75-2.62) ng/mL | | (Helmschrodt et al., 2013) |
| | | 10 ± 10 ng/mg tissue | | (Vaya et al., 2001) |
| | Carotid/aorta | 60 ± 38 ng/mg tissue | | (Garcia-Cruset et al., 2001) |
| | Coronary | 60 ± 30 ng/mg tissue | | (Vaya et al., 2001) |
| **5,6β-epoxy** | Carotid | 112.2 (95.9-334.2) ng/mg free cholesterol | – Oxidative stress (alone/mixture)<br>– Inflammation (alone/mixture)<br>– Apoptosis (alone/mixture) | (Helmschrodt et al., 2013) |
| | | 6.02 (2.68-13.03) ng/mL | | (Helmschrodt et al., 2013) |
| | Carotid/aorta | 30 ± 10 ng/mg tissue | | (Vaya et al., 2001) |
| | Coronary | 34 ± 16 ng/mg tissue | | (Garcia-Cruset et al., 2001) |
| | | 120 ± 70 ng/mg tissue | | (Vaya et al., 2001) |
| **Triol** | Carotid | 1.42 (0.76-8.05) ng/mL | – Oxidative stress<br>– Inflammation | (Helmschrodt et al., 2013) |
| | | | – Apoptosis<br>– Endothelial dysfunction/cell phenotype changes (alone)<br>– Vascular calcification | |
| **5,6-secosterol** | ND | ND | – Endothelial dysfunction/cell phenotype changes (alone) | (Gargiulo et al., 2015) |
| **Oxysterol mixture** | ND | ND | – Vascular calcification | (Gargiulo et al., 2015) |
| ***HYPERTENSION*** | | | | |
| **7α-OH** | Arteries (long-term high blood pressure could also affect heart, kidney and eyes) | 0.37 ± 0.33 μg/mL | – High blood pressure. (Long-term high pressure is also a major risk of stroke, heart failure, vision loss, peripheral vascular disease and chronic kidney disease.) (NIH U.S. National Library of NIH, 2017) | (Menendez-Carreno et al., 2011) |
| **7β-OH** | | 0.29 ± 0.24 μg/mL | | (Menendez-Carreno et al., 2011) |
| **7-keto** | | 0.08 ± 0.06 μg/mL | | (Menendez-Carreno et al., 2011), (Medicine, 2017) |
| **5,6α-epoxy** | | 0.05 ± 0.04 μg/mL | | (Menendez-Carreno et al., 2011), (Medicine, 2017) |
| **5,6β-epoxy** | | 0.06 ± 0.05 μg/mL | – Heart attack and/or stroke (due to the inactivation of the production of nitric oxide free radical).<br>– High blood pressure. (Long-term high pressure is also a major risk of stroke, heart failure, vision loss, peripheral vascular disease and chronic kidney disease.) (NIH U.S. National Library of NIH, 2017) | (Menendez-Carreno et al., 2011), (Medicine, 2017) |
| **Triol** | | .04 ± 0.03 μg/mL | – High blood pressure. (Long-term high pressure is also a major risk of stroke, heart failure, vision loss, peripheral vascular disease and chronic kidney disease.) (NIH U.S. National Library of NIH, 2017) | (Menendez-Carreno et al., 2011), (Medicine, 2017) |
| ***ONSET OF ISCHEMIC STROKE*** | | | | |
| **27-OH** | Brain artery | ~30 ng/mL | – Stop of oxygen and nutrients flow to the brain, causing a stroke. | (Lee et al., 2009), (Center) |

(*continued on next page*)

*Figure 6: (cont'd)*



**Table 2** (*continued*)

| | | | | |
|---|---|---|---|---|
| **24S-OH** | | ~50 ng/mL | | (Lee et al., 2009) |
| **7α-OH** | | ~25 ng/mL | | (Lee et al., 2009) |
| **7β-OH** | | ~4.2 ng/mL | | (Lee et al., 2009), (Center) |
| **7-keto** | | ~30 ng/mL | | (Lee et al., 2009), (Center) |
| **NEUROLOGICAL DISEASES** | | | | |
| ***HUNTINGTON'S DISEASE*** | | | | |
| **Oxysterol** | **Affected Tissue** | **Concentration exerting biological activity** | **Effect** | **Reference** |
| **27-OH** | Brain | 154.2-189.1 ± (33.5-53.1) μg/mL | – Neuronal dysfunction and death (due to transcriptional repression, proteasome, impairment, oxidative injury and mitochondrial dysfunction) | (Leoni et al., 2011) |
| **24S-OH** | Brain | 47.83 ± 64.57 μg/mL | – Neuronal dysfunction and death (due to transcriptional repression, proteasome impairment, oxidative injury and mitochondrial dysfunction) | (Leoni et al., 2013) |
| | | 46.0-56.9 ± (10.4-8.4) μg/mL | | (Leoni et al., 2008) |
| | | 42.7-61.0 ± (9.7-11.9) μg/mL | | (Leoni et al., 2011) |
| ***PARKINSON'S DISEASE*** | | | | |
| **27-OH** | Brain | ~90 ng/mL | – Aggregation of α-synuclein protein in Lewy body inclusions.<br>– Death of dopaminergic neurons in the *substantia nigra*. | (Lee et al., 2009), (Marwarha and Ghribi, 2015), (Marwarha and Ghribi, 2015) |
| **24S-OH** | | ~10 ng/mL | | (Lee et al., 2009), (Marwarha and Ghribi, 2015) |
| **7α-OH** | | ~29 ng/mL | | (Lee et al., 2009) (Marwarha and Ghribi, 2015) |
| **7β-OH** | | ~10 ng/mL | | (Lee et al., 2009), (Marwarha and Ghribi, 2015) |
| **7-keto** | | ~55 ng/mL | | (Lee et al., 2009) |
| ***MULTIPLE SCLEROSIS*** | | | | |
| **27-OH** | Central nervous system (neurons) | 21 ± 114 ng/mL | – Damage to the myelin in the central nervous system. | (Mukhopadhyay et al., 2017) |
| **24S-OH** | | ~35.3 ± 20 ng/mL | | (Mukhopadhyay et al., 2017) |
| **25-OH** | | 18.6 ± 38 ng/mL | | (Mukhopadhyay et al., 2017) |
| **7α-OH** | | 69.9 ± 74 ng/mL | | (Mukhopadhyay et al., 2017) |
| **7-keto** | | 18.1 ± 17 ng/mL | | (Mukhopadhyay et al., 2017) |
| ***ALZHEIMER'S DISEASE*** | | | | |
| **24S-OH** | Central nervous system | 75 ± 18 ng/mL | – Formation of protein aggregates in the human brain, extracellular deposition of Aβ in senile plaques<br>– Intracellular accumulation of hyperphosphorylated Tau (Tubulin Associated Unit) in neurofibrillary tangles (NFT)<br>– Neuron degeneration and synaptic loss | (Lutjohann et al., 2000), (Philip Scheltens and Giovanni B Frisoni, 2006) |
| **VIRAL INFECTIONS** | | | | |
| ***ONSET DENGUE FEVER (MOSQUITO-BORNE VIRAL INFECTION)*** | | | | |
| **27-OH** | Immunological system | ~38 ng/mL | – Oxidative stress<br>– Infection of the Fc receptor–bearing cells such as monocyte/macrophages (which activates the precursor cross-reactive memory T cells, increasing disease severity) | (Lee et al., 2009) |
| | | 41.5 ± 19.8 ng/mL | | (Seet et al., 2009), (Vaughn et al., 1997) |
| **24S-OH** | | ~15 ng/mL | | (Lee et al., 2009) |

(*continued on next page*)

*Figure 6:* *(cont'd)*



**Table 2** (*continued*)

| | | | | |
|---|---|---|---|---|
| | | 18.1 ± 5.6 ng/mL | | (Vaughn et al., 1997), (Seet et al., 2009) |
| **7α-OH** | | ~12 ng/mL | | (Vaughn et al., 1997) (Lee et al., 2009) |
| **7β-OH** | | ~4 ng/mL | | (Vaughn et al., 1997), (Lee et al., 2009) |
| | | 4.3 ± 2.2 ng/mL | | (Seet et al., 2009) |
| **7-keto** | | ~25 ng/mL | | (Vaughn et al., 1997), (Lee et al., 2009) |
| ***HEPATITIS C*** | | | | |
| **27-OH** | Liver | 138.6 ± 6.3 ng/mL | – Oxidative stress and dysregulated cholesterol metabolism.<br>– Primary risk factor for liver cancer.27-OHC also increased prostate-specific antigen expression and enhanced AR binding to the androgen response element compared to controls. | (Ikegami et al., 2014; Raza et al., 2016)(Ikegami et al., 2014; Raza et al., 2016)(Ikegami et al., 2014; Raza et al., 2016) |
| **24S-OH** | | 64.6 ± 2.0 ng/mL | – Oxidative stress and dysregulated cholesterol metabolism.<br>– Primary risk factor for liver cancer. | (Ikegami et al., 2014) |
| **25-OH** | | 21.4 ± 0.8 ng/mL | | (Ikegami et al., 2014) |
| **7α-OH** | | 458.6 ± 18.6 ng/mL | | (Ikegami et al., 2014) |
| **CANCER** | | | | |
| ***BREAST CANCER*** | | | | |
| **27-OH** | Breast | 1-2 μmol/L | – Promotes the proliferation of the estrogen receptor (ER) positive breast cancer cell lines in vitro. | (Cruz et al., 2010) |
| | | $10^{-8}$ – $10^{-6}$ mol/L | | (Wu et al., 2013) (Nelson et al., 2014) |
| **25-OH** | | 1 μmol/L | – 25HC induce the recruitment of ERa to the ERE site located in the pS2 promoter sequence in MCF7 cells. | (Lappano et al., 2011) |
| | | | – Induce proliferative effects in a dose dependent manner. | |
| ***PROSTATE CANCER*** | | | | |
| **27-OH** | Prostate | 0.1 and 1.0 μmol/L | – Stimulates proliferation and increases androgen receptor (AR) transcriptional activity.<br>– 27-OHC also increased prostate-specific antigen expression and enhanced AR binding to the androgen response element compared to controls. | (Raza et al., 2016) |
| ***OVARIAN CANCER*** | | | | |
| **25-OH** | Ovaries | 1 μmol/L | – Induce proliferative effects in a dose dependent manner. | (Lappano et al., 2011; Raza et al., 2016)(Lappano et al., 2011; Raza et al., 2016)(Lappano et al., 2011; Raza et al., 2016) |
| ***LUNG CANCER*** | | | | |
| **7α-OH** | Lung | 163.4 ± 107.9 nmol/L | – Inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, a key enzyme in the endogenous cholesterol synthesis pathway. | (Linseisen et al., 2002; Raza et al., 2016)(Linseisen et al., 2002; Raza et al., 2016)(Linseisen et al., 2002; Raza et al., 2016)<br>(Kang et al., 2005; Lappano et al., 2011) |
| **7β-OH** | | 56.3 ± 15.2 nmol/L | | (Linseisen et al., 2002), (Kang et al., 2005) |
| **7-keto** | | 99.0 ± 43.3 nmol/L | – Inhibitor of DNA synthesis. | (Sevanian and Peterson, 1984), (Linseisen et al., 2002) |
| **5,6α-epoxy** | | 273.5 ± 314.9 nmol/L | – Inhibitor of DNA synthesis. (Sevanian and Peterson, 1984) | (Sevanian and Peterson, 1984), (Linseisen et al., 2002) |

*Figure 6: (cont'd)*



a large number of deaths has been caused by chronic diseases some of them closely related with COPs.

From a physiological point of view, individual COPs play an important role in the development of several age-related diseases because of a decrease in oxidative defenses and control of the level of oxysterols, as it is observed in hypercholesterolemia (Micheletta et al., 2004; Zarrouk et al., 2014). Hypercholesterolemia not only can result in coronary heart disease, stroke, and peripheral arterial disease, type 2 diabetes, and hypertension atherosclerosis but high amounts of cholesterol in the body increase the amounts of COPs in the body as well (Carpenter et al., 2003; Shim et al., 2008; Garcia-Cruset et al., 2001; Vaya et al., 2001; Gargiulo et al., 2015; Menendez-Carreno et al., 2011; Murakami et al., 2001; Upston et al., 2002; NIH, 2017). Given the different biochemical and biophysical properties of COPs in altering specific cell compartments and enzyme/receptors, several cells and tissue-specific pathological effects have been reported. Different studies have shown that some COPs adversely affect the function of some major organs like brain, eyes, heart and vessels (Bretillon et al., 2007; Gargiulo et al., 2016). Understanding how the structure and chemistry of COPs define their specific function and mechanism(s) of action inside the human body, could help to identify how COPs affect targeted tissues and the exact conditions that triggers their formation. Many hypotheses have arisen to explain the specific role that COPs play in the pathology of a variety of diseases (Table 2).

### 2.3. COPs as biomarkers of chronic diseases

Several experimental evidences point out that COPs can adversely affect the function of some major organs, and the interaction and mechanisms of COPs in the specific affected organ is still not well understood. Lee et al (2009) have suggested that the apparent association of specific COPs with people more prone to suffer of these chronic diseases could be beneficial, as COPs could be used as biomarkers for these diseases (Iuliano et al., 2003; Aldini et al., 2010; Alkazemi et al., 2008). As an example, Linseisen's work (Linseisen et al., 2002) associated the presence of 7β-OH with lung cancer risk revealing high risk estimates, which was lately confirmed by Kang and co-workers (Kang et al., 2005). Abnormal cholesterol biosynthesis has been associated to tissue and fluid accumulation of 7β-OH in Smith-Lemli-Optiz syndrome (Xu et al., 2011, 2012). Yoshida et al., (2003) found some sort of connection between oxysterols and the hepatic bile disease, proposing that the most common oxysterols in gallstones may be generated in the gallbladder in response to bacterial infection. Leoni and Caccia (2011) mentioned the use of 24S-OH as a possible surrogate biomarker by the number of metabolically active neurons located in the grey matter of the brain due to its reduced levels of 24-hydroxylase (CYP46A1) with subsequent reduction in the formation of 24S-OH and lower efflux from the brain to the circulation (Leoni et al., 2008, 2013). These levels of 24S-OH in the circulation were found to be significantly reduced compared to controls in different neurodegenerative diseases such as Alzheimer's disease (AD), Multiple sclerosis (MS) and Huntington's disease (HD) (Lutjohann et al., 2000; Leoni and Caccia, 2011; Leoni et al., 2011). They also associated higher levels of 27-OH with the processing and deposition of Aβ peptides in both AD and Mild Cognitive Impairment (MCI) patients. In individuals suffering multiple sclerosis (MS), elevated concentrations of 7-keto has been detected in the cerebrospinal fluid; however, mechanistic evidences have been reported only for the induction of neuronal damage *via* the activation and migration of microglial cells (Vejux and Lizard, 2009). Other COPs derived from cholesterol auto-oxidation (7α-OH, 7β-OH, and 25-OH) also present in the central nervous system, are known to cause damage to the myelin; however, a complete understanding of the associated molecular mechanisms is still missing (Mukhopadhyay et al., 2017). Huntington's disease (HD), a disease that causes neuronal dysfunction and death, has been associated to transcriptional repression, oxidative injury and mitochondrial dysfunction provoked by accumulation of enzymatic 24S-OH and 27-OH in brain. Analyses performed *post mortem* on brain tissue of HD patients showed a 60% decrease in 24S-OH, 30% increase in cholesterol, and 50–70% increase in 7-keto and 7β-OH (both derived predominantly from ROS action on cellular cholesterol), suggesting a significant inhibition of cholesterol metabolism and the contribution of oxidative stress in HD pathology (Kreilaus et al., 2016). Lastly, for Parkinson's disease, Bosco et al. (2006) stated that enzymatic oxysterols (mainly 24S-OH and 27-OH, and secosterol) are particularly responsible for causing α-synuclein aggregation and destruction of dopamine-containing neurons. All these relationships could help to use COPs as biomarkers in different human diseases. Nevertheless, the concurrence of biochemical action (i.e. cytochrome P450s metabolism) and oxidative stress (i.e. cholesterol auto-oxidation) in the formation of individual COPs make challenging a severe and robust identification of disease-specific markers. Thus, although a potential use of COPs as biomarker exists, this cannot prescind from an overall assessment of individual physiology and oxidative conditions.

### 2.4. COPs as promoter of cellular apoptosis

Even though there is a knowledge gained over the years about the mechanisms by which COPs exert their pathologic effects, and considering that cholesterol oxidation generates more than 70 derivatives, volatile organics and $H_2O_2$ (Smith, 1996), we still lack an exhaustive comprehension of the mechanisms of formation and action. It is worth to mention that there is a significant overlap between COPs generated *via* enzymatic pathways (mainly P450's associated metabolism of cholesterol and other COPs precursors) and COPs derived from auto-oxidation of cholesterol (Table 1).

There is a copious amount of literature demonstrating COPs' cytotoxicity on several cell lines and *in vivo*. The cytotoxic activity of COPs is mainly derived from their ability to induce apoptosis through several mechanisms. An exhaustive discussion of biological action of COPs is beyond the scope of this review, and the authors suggest a few compelling reviews already published. Here, we will summarize the significant findings regarding the role played by those COPs which are derived from diet.

Apoptosis is a programmed cell death, a critical biological process involved in ontogenesis and tissue homeostasis. Dysregulation of apoptosis can promote important diseases, including cancer and atherosclerosis (Lopez and Tait, 2015; Seimon and Tabas, 2009). In general terms, cell apoptosis occurs via two pathways: the mitochondrial or *intrinsic* pathway and the death receptor-dependent or *extrinsic* pathway (Leonarduzzi et al., 2007; Lordan et al., 2009). The first leads to direct activation of caspases, a family of cytosolic proteases that transmit the apoptotic pathway by making specific protein cleavages (Wolf and Green, 1999). On the other hand, the mitochondrial pathway involves alterations of the mitochondrial potential, which triggers the production of ROS and/or mitochondrial membrane permeabilization (Biasi et al., 2009; Leonarduzzi et al., 2007). Mostly, endogenous COPs mediate apoptosis via the mitochondrial pathway, although a few recent studies have found evidences of actions on the caspase cascade as well (Lordan et al., 2009).

The death receptor pathway is activated through the binding of cytokine ligands to receptors of the tumor necrosis factor (TNF) superfamily, such as Fas, lymphotoxin, TNF receptor (TNFR) or TNF-related apoptosis-inducing ligand (Lordan et al., 2009). Both 7β-OH and 25-OH up-regulated Fas expression and induced apoptosis in vascular smooth cells when treated with oxidized LDL (oxLDL) (Lee and Chau, 2001). However, 7β-OH and 7-keto failed to induce the proapoptotic ligand TNF-α in human umbilical venous endothelial cells (HUVECs), but triggered apoptosis by activating the interleukin IL-1β secretion only after 24 h of incubation, suggesting a time-dependent increase after exposure (Lemaire et al., 1998). 7β-OH, 7-keto and 5,6β-epoxide also induced the release of the inflammatory cytokine IL-8 in U937 (human promonocytic leukemia cells), monocytes/macrophages,

*Figure 6: (cont'd)*



inasmuch as THP-1 cells (Lemaire-Ewing et al., 2005; Liu et al., 1997). Several protein kinases involved in the upstream induction and downstream execution stages of apoptosis have shown modified activity after exposure to 7β-OH and 7-keto (Adamczyk et al., 1998; Berthier et al., 2005). The activity of protein kinase C – a key enzyme of the cell activation pathway (Moog et al., 1991) - is reduced by exposure of 7β-OH in neuronal cells (Lordan et al., 2009), but not in macrophages (Moog et al., 1991), indicating a cell dependency in protein kinases' regulations.

More interesting is the potential dysregulation of mitochondrial control exerted by several COPs, since it is more directly involved with oxidative stress. 7β-OH, 7-keto, and 5,6β-epoxide has been shown to induced loss of mitochondrial transmembrane potential (Lemaire-Ewing et al., 2005), which is accompanied by release of cytochrome *c* in the cytosol (Lizard et al., 2000). Cytochrome *c*, an essential hemoprotein of the respiratory chain, has an intermediate role in apoptosis by triggering caspase 9, which in turn activates caspase 7 and 3 (Wolf and Green, 1999). The cytochrome *c* apoptotic pathway has been observed in several cell lines, including U937 (Lizard et al., 2000), MCF-7 and MCF-7/c3 cells (Prunet et al., 2005), upon exposure of 7-keto and 7β-OH. Regarding caspase activation, COPs activate both *initiator* and *effector* caspases, thus exerting activity at both up and downstream steps of the apoptotic process. Several reports have put 7-keto, 7β-OH and 25-OH in the frontline for activation of caspase 8 and 12 (initiator caspases), as well as caspase 3 (effector caspase) (Lee and Chau, 2001). Interestingly, several studies agree that the isomer 7α-OH has no effect on the caspase cascade (Lemaire-Ewing et al., 2005; Lemaire et al., 1998; Lordan et al., 2009; Wolf and Green, 1999).

Other mechanisms of cytotoxicity have been reported, including altered transport of small molecules via alteration of plasma membrane fluidity and permeabilization, cell detachment, leakage of cell enzymes, as well as interference with DNA synthesis (Table 2). The mutagenic activities of COPs have been known since the early '80s (Jusakul et al., 2011; Sevanian and Peterson, 1984, 1986). The 5,6-epoxy isomers and the derived triol have demonstrated inhibition activity towards DNA synthesis in hamster V79 cells at micromolar concentrations (Sevanian and Peterson, 1984); similar results have been reported for the 7-keto, whose effect are synergic with the epoxides (Sevanian and Peterson, 1986). It is believed that the mutagenetic effect relates to the ability of epoxides of generating ROS (Jusakul et al., 2011). Other potential procarcinogenic effects, including DNA fragmentation, have been observed in Caco-2 cells colon cancer by 7α-OH, 7β-OH and 5,6α-epoxy (Biasi et al., 2013; Roussi et al., 2005). In prostate cancer, 27-OH can induce DNA damage, regulate cyclooxygenase-2 expression, and stimulation of tumor cell migration (Cruz et al., 2010; Zarrouk et al., 2014), which is accompanied by proliferation, stimulation and increase of androgen receptor (AR) transcriptional activity (Raza et al., 2016).

### 2.5. COPs and cell survival mechanisms

Concurrently with pro-apoptotic triggering, COPs have been lately associated with survival anti-apoptotic mechanisms (Vurusaner et al., 2016a, 2016b). A study by Berthier and co-workers (Berthier et al., 2005) proved that 7-keto can delay the apoptotic effect induced by 7-keto itself at relatively high concentrations (100 μM). Indeed, 7-keto seemed to transiently induce the MAPK-Erk kinase-1 and 2 survival pathway (MEK 1/2 → ERK 1/2), inhibiting THP-1 human monocytes death by phosphorylating the Bcl-2 antagonist of cell death (BAD), which then delays mitochondrial damage (Leonarduzzi et al., 2010). A concentration-dependent behavior in U937 macrophages was also found for the side-chain enzymatic 27-OH, derived from CYP27A1 activity (Vejux and Lizard, 2009). At low concentration (2 μg/mL), 27-OH inactivates the PI3K/Akt survival cascade via phosphorylation of $Thr^{308}$ residue in Akt, whereas at higher concentrations (40 μg/mL) rapidly triggers lysosomal-independent apoptosis (Riendeau and Garenc, 2009). An analogue concentration-dependent survival signaling was observed for 7β-OH in HUVEC cells by activation of the MEK/ERK cascade (Trevisi et al., 2010).

## 3. Dietary COPs: occurrence in food

Historically, the evaluation of food quality and safety during processing, preservation, and storage has relayed in targeted single response studies, evaluating quality aspects after a particular treatment (i.e. increase stability, improve texture, flavor and digestibility), or mainly been focused in microbial reduction. On the other hand, lipid and cholesterol degradations are complex phenomena that can be addressed only by multi-response analysis, which is hard to achieve considering the intrinsic difficulties of monitoring several molecules possessing different chemical nature. Although several reports have been published in the last decades (Baggio and Bragagnolo, 2006; Brzeska et al., 2016; Derewiaka and Obiedziński, 2010; Medina-Meza and Barnaba, 2013; Olkkonen et al., 2017; Sarantinos, 1993; Savage et al., 2002; Sieber, 2005; Zardetto et al., 2014), the information regarding COPs content in foods is still incomplete. The limited data currently available has been obtained from studies performed in selected type of foods such as meat (Derewiaka and Obiedziński, 2009; Khan et al., 2015; Rey et al., 2004; Serra et al., 2016), eggs (Boselli et al., 2001; Mazalli and Bragagnolo, 2009; Tsai and Hudson, 2006), milk (Liu et al., 2016; Rose-Sallin et al., 1995; Sieber, 2005), infant formula (Przygonski et al., 2000; Romeu-Nadal et al., 2007) and few others. It is imperative to provide a database of COPs concentrations for the major food products consumed by US population, with data obtained from real cooking procedures and by different population groups.

### 3.1. Food processing triggers COPs formation in foods

Traditional food processing methods, such as drying, frying, steaming and canning, involve heat treatment of the food matrix. It is well known for several macronutrients, including amino acids, carbohydrates as well as lipids, that heat conveys sufficient energy to trigger autoxidation, causing the formation of several degradation compounds. For example, acrylamide and malondialdehyde are end-products of Maillard reaction and fatty acids lipid peroxidation, respectively. For their toxicity towards humans and their occurrence in foods, both have been classified as *hazardous substances*, and their risk exposure in humans has been constantly monitored in both United States and Europe (Dorne et al., 2009). Similarly, cholesterol oxidation gives rise to several degradation products whose toxicity has gathered the attention of the scientific community worldwide. Given the susceptibility of the C5-C6 double bond to a radical attack, heat triggers the cholesterol oxidation at temperatures as low as 100 °C (Medina-Meza et al., 2011; Min et al., 2015). These temperatures are easily achievable with common processing and food preparation techniques. Industrial processing (i.e., cooking, pasteurizing, canning, etc.) occurs at variable times and temperatures depending on the type of process and food. Effective canning of meats for example, takes place at 121 °C. Industrial frying takes place between 163 and 188 °C, depending on the type of meat; whereas in spray drying, a technique used to produce powders from a liquid, often used in the manufacturing of baby formulas, temperatures between 200 and 400 °C are used (Meister et al., 2000; Verardo et al., 2016). As a matter of facts, it has been found that several cooking methods considerably increase the formation of COPs in meats (Broncano et al., 2009; Roldan et al., 2014). Microwave heating (Herzallah, 2005), pan roasting, oven grilling (Khan et al., 2015), oil-frying (Derewiaka and Obiedziński, 2010) and other forms of cooking (Nielsen et al., 1996b) all affect the production of COPs. According to the US Department of Health and Human Services, meat and poultry should be roasted at a minimum of 162 °C (FoodSafety, 2017). Even proper roasting temperatures are more than sufficient to produce COPs (Shozen et al., 1995). Additionally, a variety of non-traditional methods of processing,

## *Figure 6:* *(cont'd)*



**Table 3**
COPs occurrence in meats and poultry.

| COPs detected | Type of meat | Process parameters | Quantity | Reference |
|---|---|---|---|---|
| **7-keto** | Beef | – Grilling and/or heat (3 hours):<br>• blue<br>• charcoal radiation | – 2.5 μg/g lipid (blue heat)<br>– 2.4 μg/g lipid (coal heat) | (Al-Ismail, 2002) |
| | | – Ground beef<br>• Raw patties stored for 4 days at 4 °C<br>• Cooked patties stored for 4 days at 4 °C | – 0.206–0.604 μg/g meat (raw)<br>– 3.92–6.22 μg/g meat (cooked) | (Vore, 1988) |
| | | – Ground beef<br>• Freshly cooked<br>• Freshly cooked, storage (4 days) at 4 °C | – 3.15 ± 0.2 μg/g meat (freshly cooked)<br>– 16.00 ± 1.00 μg/g meat (freshly cooked, storage (4 days) at 4 °C) | (Britt et al., 1998) |
| | Chicken | – Cutlet<br>– Smoked | – 2.00 ± 0.65 μg/g meat (cutlet)<br>– 2.25 ± 0.66 μg/g meat (smoked) | (Chiu et al., 2018) |
| | Chicken (commercial) | Storage (freeze-dried) | 22 ppm | (Sander et al., 1989b) |
| | Chicken shawarma | – Grilling and/or heat (3 hours):<br>• blue<br>• charcoal radiation | – 4 μg/g lipid (blue heat)<br>– 4.1 μg/g lipid (coal heat) | (Al-Ismail, 2002) |
| | Commercial jameed | Storage (7 months) at 25 °C | 43.8 μg/g | (Al-Ismail et al., 2007) |
| | Crackling emulsion without antioxidants | – Added antioxidants:<br>• CEH-enzymatic hydrolysate<br>• CAH-acidic hydrolysate<br>– BHT-Butylated Hydroxytoluene | – Control: 177 μg/mg<br>– CEH: 32.6 μg/mg<br>– CAH: 47.5 μg/mg<br>– BHT: 37.7 μg/mg | (Rudzińska et al., 2006) |
| | Ham | – Raw<br>– Roast | – 1049± 390 μg/100 g meat (raw)<br>– 1204 ± 61 μg/100 g meat (roast) | (Osada et al., 2000) |
| | Dry-cured ham | – High pressure processing (600 and 900 MPa) | – 48.42–149.48 μg/g lipid | (Clariana and Garcia-Regueiro, 2011) |
| | | – Iberian (montanera)<br>– Iberian (concentrate feed)<br>– Iberian x Duroc (montanera)<br>– Iberian x Duroc (concentrate feed) | – 71.21 ± 3.20 μg/100 g muscle (Iberian, montanera)<br>– 61.94 ± 3.49 μg/100 g muscle (Iberian, concentrated feed)<br>– 56.61 ± 1.59 μg/100 g muscle (Iberian x Duroc, montanera)<br>– 62.40 ± 5.65 μg/100 g muscle (Iberian x Duroc, concentrate feed) | (Petrón et al., 2003) |
| | Sausage | N/A | – 1.74 ± 0.79 μg/g meat | (Chiu et al., 2018) |
| | | – Pig feet skin<br>– Pig feet juice<br>– Pig feet meat | – 165.6 ± 4.2 ng/g sample (pig feet skin at 24 hours)<br>– 423.4 ± 80.2 ng/g sample (pig feet juice at 24 hours)<br>– 1134.0 ± 60.0 ng/g sample (pig feet juice-control) | (Chen et al., 2012) |
| | Minced veal | – Raw<br>– Cooked | – 0.92 ± 0.03 μg/g meat (raw)<br>– 0.2.25 ± 0.20 μg/g meat (cooked) | (Pie et al., 1991) |
| | Meat batter | Raw (irradiated, 4.5 kGy) | 14.5 μg/g meat | (Jo et al., 1999) |
| | Pork | – Boiled<br>– Cutlet | – 2.10 ± 0.25 μg/g meat (boiled)<br>– 1.35 ± 0.80 μg/g meat (cutlet) | (Chiu et al., 2018) |
| | | – Roast<br>– Bacon | – 814.6 ± 97.6 μg/100 g meat (roast)<br>– 708.3 ± 136.6 μg/100 g meat (bacon) | (Osada et al., 2000) |
| | | – Raw<br>– Cooked | – 0.92 ± 0.03 μg/g meat (raw)<br>– 2.25 ± 0.20 μg/g meat (cooked) | (Pie et al., 1991) |
| | Salami sausage (commercial) | NS | 890.2 ± 131.7 μg/100 g meat | (Osada et al., 2000) |
| | Sausage (commercial) | NS | 729.3 ± 48.8 μg/100 g meat | |
| | Turkey (commercial) | Storage (freeze-dried) | 20 ppm | (Sander et al., 1989b) |
| | Salame Milano (commercial) | NS | 2.56 μg/g sample | (Novelli et al., 1998) |
| | Mortadella (commercial) | NS | 18.69 μg/g sample | |
| | Bovine liver (commercial) | Raw | 13.8 μg/g meat | (Park and Addis, 1985) |
| **25-OH** | Dry-cured ham | High pressure processing (600 and 900 MPa) | 0.38–0.72 μg/g lipid | (Clariana and Garcia-Regueiro, 2011) |
| | | – Iberian (montanera)<br>– Iberian (concentrate feed)<br>– Iberian x Duroc (montanera)<br>– Iberian x Duroc (concentrate feed) | – tr (Iberian, montanera)<br>– tr (Iberian, concentrated feed)<br>– ND (Iberian x Duroc, montanera)<br>– ND (Iberian x Duroc, concentrate feed) | (Petrón et al., 2003) |
| | Minced veal | – Raw<br>– Cooked | – 0.13 ± 0.01 μg/g meat (raw)<br>– 0.38 ± 0.01 μg/g meat (cooked) | (Pie et al., 1991) |
| | Salame Milano (commercial) | NS | 2.64 μg/g sample | (Novelli et al., 1998) |
| | Pork | – Minced pork<br>• Raw<br>• Cooked | – 0.13 ± 0.01 μg/g meat (raw)<br>– 0.38 ± 0.01 μg/g meat (cooked) | (Pie et al., 1991) |
| | | – Boiled<br>– Cutlet | – 2.12 ± 0.14 μg/g meat<br>– 1.69 ± 0.96 μg/g meat | (Chiu et al., 2018) |
| | | – Pig feet skin<br>– Pig feet juice<br>– Pig feet meat | – 48.3 ± 0.6 ng/g sample (pig feet skin at 24 hours)<br>– 24.4 ± 1.1 ng/g sample (pig feet juice at 24 hours)<br>– 42.0 ± 1.6 ng/g sample (pig feet juice-control) | (Chen et al., 2012) |
| | Sausage | N/A | 2.16 ± 0.58 μg/g meat | (Chiu et al., 2018) |
| | Chicken | – Cutlet<br>– Smoked | – 1.75 ± 0.87 μg/g meat<br>– 2.19 ± 0.09 μg/g meat | (Chiu et al., 2018) |

(*continued on next page*)

*Figure 6:* *(cont'd)*



**Table 3** (*continued*)

| COPs detected | Type of meat | Process parameters | Quantity | Reference |
|---|---|---|---|---|
| **7α -OH** | Dry-cured ham | – High pressure processing (600 and 900 MPa) | – 9.25–31.33 μg/g lipid | (Clariana and Garcia-Regueiro, 2011) |
| | | – Iberian (montanera) | – 42.91 ± 3.26 μg/100 g muscle (Iberian, montanera) | (Petrón et al., 2003) |
| | | – Iberian (concentrate feed) | – 36.25 ± 2.43 μg/100 g muscle (Iberian, concentrated feed) | |
| | | – Iberian x Duroc (montanera) | – 37.28 ± 4.07 μg/100 g muscle (Iberian x Duroc, montanera) | |
| | | – Iberian x Duroc (concentrate feed) | – 44.27 ± 4.56 μg/100 g muscle (Iberian x Duroc, concentrate feed) | |
| | Minced veal | – Raw | – 0.18 ± 0.01 μg/g meat (raw) | (Pie et al., 1991) |
| | | – Cooked | – 0.64 ± 0.01 μg/g meat (cooked) | |
| | Meat batter | – Raw (unirradiated) | – 17.6 μg/g meat (unirradiated) | (Jo et al., 1999) |
| | | – Raw (irradiated, 4.5 kGy) | – 72.6 μg/g meat (irradiated, 4.5 kGy) | |
| | Pork | – Minced pork | – 0.19 ± 0.01 μg/g meat (raw) | (Pie et al., 1991) |
| | | • Raw | – 0.64 ± 0.01 μg/g meat (cooked) | |
| | | • Cooked | | |
| | | – Boiled | – 2.28 ± 0.23 μg/g meat | (Chiu et al., 2018) |
| | | – Cutlet | – 2.06 ± 0.10 μg/g meat | |
| | | – Pig feet skin | – 97.3 ± 0.1 ng/g sample (pig feet skin at 24 hours) | (Chen et al., 2012) |
| | | – Pig feet juice | – 135.7 ± 14.1 ng/g sample (pig feet juice at 24 hours) | |
| | | – Pig feet meat | – 464.6 ± 27.3 ng/g sample (pig feet juice-control) | |
| | Sausage | N/A | 2.24 ± 0.19 μg/g meat | (Chiu et al., 2018) |
| | Chicken | – Cutlet | – 2.04 ± 0.09 μg/g meat | |
| | | – Smoked | – 2.43 ± 0.55 μg/g meat | |

**Abbreviations**: tr = trace, N/A = not applicable, ND = Not Determined, NS = Not Specified.

including infrared heating, microwave heating, ohmic heating, high pressure processing, ionizing radiation, pulsed electric field and ultrasound are believed to be less harsh on meat products (Avsaroglu et al., 2006; Medina-Meza et al., 2014a,b). Compared to traditional technologies, these novel thermal and nonthermal technologies reduce/eliminate temperature exposure or decrease treatment time, but do not eliminate oxidation triggering factors all together (Herzallah, 2005; Medina-Meza and Barnaba, 2013; Medina-Meza et al., 2014a,b). However, we are far from a conclusive evidence that those novel technologies are reliable alternatives to lipid and cholesterol degradation (Medina-Meza et al., 2014a,b). Prepared meals – like ready-to-eat foods – also pose a significant risk (Ubhayasekera et al., 2006). These meals usually undergo several processing stages, from heating to pasteurization temperatures, followed by freezing, as part of the storage process before distribution; once stored for days, ready-to-eat meals finally make it home where they are re-heated or microwaved. It has been found that refrigeration as well as re-heating increase the formation of COPs (Min et al., 2016).

### 3.2. Baby foods

Exposure to food-derived toxic compounds can be critical in sensitive populations, specially infants and children. Powdered milk is a known source of COPs, although the reported amounts differ according to the milk process (Dionisi et al., 1998; Leonarduzzi et al., 2005; Przygonski et al., 2000; Romeu-Nadal et al., 2007; Scopesi et al., 2002; Pickett-Bernard, 2006). Powdered milk is manufactured using the wet blending-spray drying process in cow milk. This process begins with milk pasteurization to decrease the pathogenic bacteria. The milk is then evaporated at 77 °C and then sent to the spray drying unit. This unit pre-heats the product up to 93 °C; the product is then pumped in the spray dryer at 138–204 °C. This process provides the proper conditions for triggering hydroperoxide formation that lead lipid and protein oxidation, among others reactions (Damjanovic Desic and Birlouez-Aragon, 2011; Sieber, 2005). McCluskey and co-workers found up to 60 ppm COPs in skim powdered milk (McCluskey and Devery, 1993), whereas Scopesi et al. (2002) demonstrated that the content in 7-keto is in baby formula is 5-fold higher than human breast milk. Infant formula plays an important role in infant growth and health; according to a report from the US Institute of Medicine, it may be the only source of nutrition for many infants during the first 4–6 months of life (2006). Around 2.7 million of infants by the age of three months rely on it for some portion of their nutrition (Martin et al., 2016). Infant formulations are enriched with several additives, including essential polyunsaturated fatty acids (PUFA), phytosterols, vitamins, which may play a synergistic role in the oxidation promotion of COPs. PUFAs are highly susceptible to oxidation, and hence favored the cholesterol oxidation as well (Barriuso et al., 2015; Barnaba et al., 2016; Barriuso et al., 2016). Among them, docosahexaenoic acid (DHA), and arachidonic acid (ARA) are the most common fatty acid of the PUFA family used in enriched milks, they are critical for the brain and retina development, and therefore have an influence upon visual acuity and learning abilities (Romeu-Nadal et al., 2007). As infants progress from formula to solid foods (i.e. meat, eggs, cheese, etc.), their exposure to COPs will potentially increase and affect their metabolism (Sander et al., 1989b). Zunin et al. (2006) found considerable amounts of 7-keto and triol in meat-based homogenates (2.3 g and 0.7 g per serving respectively), which was dramatically increased when vegetable oil was added to the formulations.

### 3.3. Food chain: packaging and storage

After the animal food products are processed and before they are consumed, they are packaged, shipped and stored. Packaging and storage methods can also affect the formation of oxidative products. It has also been shown that storage time can increase cholesterol oxidation (Du and Ahn, 2000; Mazalli and Bragagnolo, 2007; Nielsen et al., 1996a; Petrón et al., 2003; Pie et al., 1991; Tarvainen et al., 2015; Vore, 1988). The content of COPs has been reported to increase by six times after 2 weeks of storage at 4 °C, being higher in cooked meat than in

*Figure 6:* *(cont'd)*



**Table 4**
COPs occurrence in milk and dairy products.

| COPs detected | Matrix | Process parameters | Quantity | Reference |
|---|---|---|---|---|
| **7-keto** | Infant Formula | Fresh | 0.1–0.29 μg/g sample | (Rose-Sallin et al., 1995) |
| | | N/A | ND-18 μg/g sample | (Guardiola et al., 2002) |
| | | – Sample A | – 1.79 ± 0.45 μg/g lipid extract (Sample A) | (Przygonski et al., 2000) |
| | | – Sample B | – 2.16 ± 0.25 μg/g lipid extract (Sample B) | |
| | | – Sample C | – 1.61 ± 0.28 μg/g lipid extract (Sample C) | |
| | | – Sample D | – 3.24 ± 0.62 μg/g lipid extract (Sample D) | |
| | | – Sample E | – 2.81 ± 1.05 μg/g lipid extract (Sample E) | |
| | Milk powder | – Heating (5 minutes) | – 15.363 μg/g fat (boiled) | (Herzallah, 2005) |
| | | – Microwave at 96.3 °C | – 50.029 μg/g fat (microwaved) | |
| | | – Boil at 95.5 °C | – 80.97 μg/g fat (powder) | |
| | | Artificial light in the presence of air for 19 days | 22.415 ± 3.690 μg/g sample | (Dionisi et al., 1998) |
| | | – Skimmed milk IF powder (18 months old and opened, A) | – 915 ± 124 ng/g sample (A) | (Gorassini et al., 2017) |
| | | | – 254 ± 25 ng/g sample (B) | |
| | | – Skimmed milk IF powder (B) | – 96 ± 18 ng/g sample (C) | |
| | | – Skimmed milk powder (C) | – 34 ± 3 ng/g sample (D) | |
| | | – Instant formula for ginseng coffee (D) | – 13 ± 10 ng/g sample (E) | |
| | | – Instant formula for cream coffee (E) | | |
| | | Full cream (stored for 3–12 months) | < 0.1 μg/g sample | (Rose-Sallin et al., 1995) |
| | | – Sample F | – 1.98 ± 0.51 μg/g lipid extract (Sample F) | (Przygonski et al., 2000) |
| | | – Sample G | – 1.76 ± 0.68 μg/g lipid extract (Sample G) | |
| | | – Sample H | – 2.08 ± 0.96 μg/g lipid extract (Sample H) | |
| | | Skim milk (stored for 6 months) | 2.5 ± 0.4 μg/g sample | (Angulo et al., 1997) |
| | | – Whole milk: | | |
| | | • fresh | 0.4 ± 0.0 μg/g sample (fresh) | |
| | | • stored for 6 months at 21 and 38 °C | 2.3 ± 0.0 μg/g sample (6 months at 21 and 38 °C) | |
| | | – Skimmed | – 21.8 ng/mL sample (skimmed) | (Calderón-Santiago et al., 2012) |
| | | – For unweaned baby | – 2.4 ng/mL sample (unweaned baby) | |
| | | – Follow-on milk | – 2.9 ng/mL sample (follow-on milk) | |
| | | – Condensed milk | – 1.8 ng/mL sample (condensed milk) | |
| | UHT milk | – Full-cream | – 6.5 ng/mL sample (full-cream) | |
| | | – Skimmed | – 20.3 ng/mL sample (skimmed) | |
| | Butter | – Storage | – 5.1–8.6 μg/g sample (fresh) | (Pie et al., 1991) |
| | | • Fresh | – 0.42–0.97 μg/g sample (3–6 months, unheated) | |
| | | • 3–6 months (unheated) | – 5–14.4 μg/g sample (3–6 months, after heating for 10–20 min at 170–180 °C) | |
| | | • 3–6 months (after heating for 10–20 min at 170–180 °C) | | |
| | Powdered cheese | – Storage: | – ND-12 μg/g sample (6 months at 21 and 38 °C) | (Sander et al., 1989a,b) |
| | | • 6 months at 21 and 38 °C | – ND-39 μg/g sample (18 months at 4 °C) | |
| | | • 18 months at 4 °C | | |
| | Cheese spread | N/A | 0.48 ±0.03 μg/g sample | (Schmarr et al., 1996) |
| | Parmesan cheese | N/A | 1.22 ±0.01 μg/g sample | |
| | Cheese | – Grated | ND-0.4 μg/g sample | (Nourooz-Zadeh and Appelqvist, 1988) |
| | | – Storage (4–12 months) | | |
| | Dairy spreads | Storage (13 week) at 20 °C | 5.3 μg/g sample | (Nielsen et al., 1996a) |
| | Feta cheese | – Produced from butter oil | 1.2 μg/g sample | (Nielsen et al., 1996b) |
| | | – Storage (5 months) at 4 °C | | |
| | | Fresh | 5.5 μg/g sample | (Nielsen et al., 1995) |
| | Yellow cheese | Sliced fresh | 5.5 μg/g sample | |
| | | – Sliced, fluorescent light | 220 μg/g sample | |
| | | – Storage (55 days) at 4 °C | | |

(*continued on next page*)

*Figure 6:* *(cont'd)*



**Table 4** (*continued*)

| COPs detected | Matrix | Process parameters | Quantity | Reference |
|---|---|---|---|---|
| **7α-OH** | Infant formula | Fresh | 0.09–0.31 μg/g sample | (Rose-Sallin et al., 1995) |
| | | N/A | ND-8 μg/g sample | (Guardiola et al., 2002) |
| | | – Sample A | – 1.23 ± 0.07 μg/g lipid extract (Sample A) | (Przygonski et al., 2000) |
| | | – Sample B | – 0.72 ± 0.15 μg/g lipid extract (Sample B) | |
| | | – Sample C | – 0.65 ± 0.12 μg/g lipid extract (Sample C) | |
| | | – Sample D | – 1.42 ± 0.24 μg/g lipid extract (Sample D) | |
| | | – Sample E | – 1.20 ± 0.19 μg/g lipid extract (Sample E) | |
| | Milk powder | Full cream (stored for 3–12 months) | 0.08–0.67 μg/g sample | (Rose-Sallin et al., 1995) |
| | | – Skim milk: | – 0.1 ± 0.0 μg/g sample (fresh) | (Angulo et al., 1997) |
| | | • fresh | – 2.1 ± 0.1 μg/g sample (6 months) | |
| | | • stored for 6 months | | |
| | | – Sample F | – 0.79 ± 0.13 μg/g lipid extract (Sample F) | (Przygonski et al., 2000) |
| | | – Sample G | – 0.75 ± 0.16 μg/g lipid extract (Sample G) | |
| | | – Sample H | – 0.85 ± 0.40 μg/g lipid extract (Sample H) | |
| | | Whole milk (stored for 6 months at 21 and 38 °C) | 2.5 ± 0.0 μg/g sample (6 months at 21 and 38 °C) | (Angulo et al., 1997) |
| | | Artificial light in the presence of air for 19 days | 10.813 ± 2.620 μg/g sample | (Dionisi et al., 1998) |
| | | – Skimmed milk IF powder (18 months old and opened, A) | – 533 ± 40 ng/g sample (A) | (Gorassini et al., 2017) |
| | | | – 49 ± 10 ng/g sample (B) | |
| | | – Skimmed milk IF powder (B) | – 156 ± 28 ng/g sample (C) | |
| | | – Skimmed milk powder (C) | – 37 ± 7 ng/g sample (D) | |
| | | – Instant formula for ginseng coffee (D) | – 125 ± 16 ng/g sample (E) | |
| | | – Instant formula for cream coffee (E) | | |
| | Butter | – Storage | – 1.2–3.9 μg/g sample (fresh) | (Pie et al., 1991) |
| | | • Fresh | – ND-0.22 μg/g sample (3–6 months, unheated) | |
| | | • 3–6 months (unheated) | – 1.6–8.9 μg/g sample (3–6 months, after heating for 10–20 min at 170–180 °C) | |
| | | • 3–6 months (after heating for 10–20 min at 170–180 °C) | | |
| | Cheese spread | N/A | 0.59 ±0.02 μg/g sample | (Schmarr et al., 1996) |
| | Parmesan cheese | N/A | 1.16 ±0.05 μg/g sample | |
| | Hard cheese | – Melted | tr | (Nourooz-Zadeh and Appelqvist, 1988) |
| | | – Storage (2–4 months) | | |
| | Cheese | – Grated | ND-0.8 μg/g sample | |
| | | – Storage (4–12 months) | | |
| | Dairy spreads | Storage (13 week) at 20 °C | 1.3 μg/g sample | (Nielsen et al., 1996a) |
| | Feta cheese | – Produced from butter oil | 0.6 μg/g sample | (Nielsen et al., 1996b) |
| | | – Storage (5 months) at 4 °C | | |
| | | Fresh | 4 μg/g sample | (Nielsen et al., 1995) |
| | Yellow cheese | Sliced fresh | 1.2 μg/g sample | |
| | | – Sliced, fluorescent light | 1 μg/g sample | |
| | | – Storage (55 days) at 4 °C | | |

(*continued on next page*)

*Figure 6:* *(cont'd)*



**Table 4** (*continued*)

| COPs detected | Matrix | Process parameters | Quantity | Reference |
|---|---|---|---|---|
| **7β-OH** | Infant formula | Fresh | 0.06–0.63 μg/g sample | (Rose-Sallin et al., 1995) |
| | | N/A | ND-9 μg/g sample | (Guardiola et al., 2002) |
| | | – Sample A | – 0.92 ± 0.05 μg/g lipid extract (Sample A) | (Przygonski et al., 2000) |
| | | – Sample B | – 0.92 ± 0.18 μg/g lipid extract (Sample B) | |
| | | – Sample C | – 0.78 ± 0.18 μg/g lipid extract (Sample C) | |
| | | – Sample D | – 1.08 ± 0.17 μg/g lipid extract (Sample D) | |
| | | – Sample E | – 0.98 ± 0.41 μg/g lipid extract (Sample E) | |
| | Milk powder | Full cream (stored for 3–12 months) | 0.03–1.15 μg/g sample | (Rose-Sallin et al., 1995) |
| | | Skim milk (stored for 6 months) | 1.4 ± 0.0 μg/g sample | (Angulo et al., 1997) |
| | | – Sample F | – 0.50 ± 0.10 μg/g lipid extract (Sample F) | (Przygonski et al., 2000) |
| | | – Sample G | – 0.40 ± 0.13 μg/g lipid extract (Sample G) | |
| | | – Sample H | – 0.53 ± 0.23 μg/g lipid extract (Sample H) | |
| | | Whole milk (stored for 6 months at 21 and 38 °C) | 0.8 ± 0.0 μg/g sample (6 months at 21 and 38 °C) | (Angulo et al., 1997) |
| | | Artificial light in the presence of air for 19 days | 10.029 ± 0.759 μg/g sample | (Dionisi et al., 1998) |
| | | – Skimmed milk infant formula powder (18 months old and opened, A) | – 698 ± 47 ng/g sample (A) | (Gorassini et al., 2017) |
| | | | – 48 ± 12 ng/g sample (B) | |
| | | – Skimmed milk infant formula powder (B) | – 137 ± 28 ng/g sample (C) | |
| | | – Skimmed milk powder (C) | – 39 ± 10 ng/g sample (D) | |
| | | – Instant formula for ginseng coffee (D) | – 151 ± 13 ng/g sample (E) | |
| | | – Instant formula for cream coffee (E) | | |
| | | – Skimmed | – 206.5 ng/mL sample (skimmed) | (Calderón-Santiago et al., 2012) |
| | | – Evaporated milk | – 18.3 ng/mL sample (evaporated milk) | |
| | | – Condensed milk | – 25.6 ng/mL sample (condensed milk) | |
| | UHT milk | – Full-cream | – 81.0 ng/mL sample (full-cream) | |
| | | – Skimmed | – 101.14 ng/mL sample (skimmed) | |
| | Butter | – Storage | – 1.7–4.6 μg/g sample (fresh) | (Pie et al., 1991) |
| | | • Fresh | – 1.9–14.9 μg/g sample (3–6 months, after heating for 10–20 min at 170–180 °C) | |
| | | • 3–6 months (after heating for 10–20 min at 170–180 °C) | | |
| | Powdered cheese | – Storage: | – ND-11 μg/g sample (6 months at 21 and 38 °C) | (Sander et al., 1989a,b) |
| | | • 6 months at 21 and 38 °C | – ND-22 μg/g sample (18 months at 4 °C) | |
| | | • 18 months at 4 °C | | |
| | Cheese spread | N/A | 0.75 ±0.01 μg/g sample | (Schmarr et al., 1996) |
| | Parmesan cheese | N/A | 1.31 ±0.04 μg/g sample | |
| | Soft cheese | – Melted | ND-0.2 μg/g sample | (Nourooz-Zadeh and Appelqvist, 1988) |
| | | – Storage (6–18 months) | | |
| | Cheese | – Grated | 0.3–0.8 μg/g sample | |
| | | – Storage (4–12 months) | | |
| | Dairy spreads | Storage (13 week) at 20 °C | 1.6 μg/g sample | (Nielsen et al., 1996a) |
| | Feta cheese | – Produced from butter oil | 0.3 μg/g sample | (Nielsen et al., 1996b) |
| | | – Storage (5 months) at 4 °C | | |
| | | Fresh | 1.2 μg/g sample | (Nielsen et al., 1995) |
| | Yellow cheese | Sliced fresh | 0.9 μg/g sample | |
| | | – Sliced, fluorescent light | 1.2 μg/g sample | |
| | | – Storage (55 days) at 4 °C | | |

(*continued on next page*)

*Figure 6:* *(cont'd)*



**Table 4** (*continued*)

| COPs detected | Matrix | Process parameters | Quantity | Reference |
|---|---|---|---|---|
| **5,6α-epoxy** | Infant formula | N/A | 2–3 μg/g sample | (Guardiola et al., 2002) |
| | | – Sample A | – 2.75 ± 1.13 μg/g lipid extract (Sample A) | (Przygonski et al., 2000) |
| | | – Sample B | – 1.94 ± 0.40 μg/g lipid extract (Sample B) | |
| | | – Sample C | – 2.00 ± 0.3 μg/g lipid extract (Sample C) | |
| | | – Sample D | – 1.72 ± 1.13 μg/g lipid extract (Sample D) | |
| | | – Sample E | – 4.20 ± 0.08 μg/g lipid extract (Sample E) | |
| | Milk powder | – Skim milk: | – ND-10 μg/g sample (fresh) | (Angulo et al., 1997) |
| | | • fresh | – ND-10 μg/g sample (6 months) | |
| | | • stored for 6 months | | |
| | | – Sample F | – 2.43 ± 0.49 μg/g lipid extract (Sample F) | (Przygonski et al., 2000) |
| | | – Sample G | – 1.56 ± 0.1 μg/g lipid extract (Sample G) | |
| | | – Sample H | – 2.00 ± 0.59 μg/g lipid extract (Sample H) | |
| | | – Whole milk: | – 0.6 ± 0.0 μg/g sample (fresh) | (Angulo et al., 1997) |
| | | • fresh | – ND-10 μg/g sample (6 months at 21 and 38 °C) | |
| | | • stored for 6 months at 21 and 38 °C | | |
| | | – Skimmed milk infant formula powder (18 months old and opened, A) | – 265 ± 24 ng/g sample (A) | (Gorassini et al., 2017) |
| | | | – 39 ± 9 ng/g sample (B) | |
| | | – Skimmed milk infant formula powder (B) | – 51 ± 15 ng/g sample (C) | |
| | | – Skimmed milk powder (C) | – 18 ± 4 ng/g sample (D) | |
| | | – Instant formula for ginseng coffee (D) | – 45 ± 10 ng/g sample (E) | |
| | | – Instant formula for cream coffee (E) | | |
| | | – Skimmed milk | – 8.3 ng/mL sample (skimmed milk) | (Calderón-Santiago et al., 2012) |
| | | – Follow-on milk | – 4.3 ng/mL sample (follow-on milk) | |
| | | – Evaporated milk | – 5.3 ng/mL sample (evaporated milk) | |
| | | – Condensed milk | – 1.7 ng/mL sample (condensed milk) | |
| | UHT milk | – Full-cream | – 4.7 ng/mL sample (full-cream) | |
| | | – Skimmed | – 14.9 ng/mL sample (skimmed) | |
| | Butter | – Storage | – 0.9 2.9 μg/g sample (fresh) | (Pie et al., 1991) |
| | | • Fresh | – 1–7.4 μg/g sample (3–6 months, after heating for 10–20 min at 170–180 °C) | |
| | | • 3–6 months (after heating for 10–20 min at 170–180 °C) | | |
| | Powdered cheese | – Storage: | – ND-10 μg/g sample (6 months at 21 and 38 °C) | (Sander et al., 1989a,b) |
| | | • 6 months at 21 and 38 °C | – 2.0–16 μg/g sample (18 months at 4 °C) | |
| | | • 18 months at 4 °C | | |
| | Cheese spread | N/A | 0.68 ±0.04 μg/g sample | (Schmarr et al., 1996) |
| | Parmesan cheese | N/A | 0.93 ±0.08 μg/g sample | |
| | Hard cheese | – Melted | tr | (Nourooz-Zadeh and Appelqvist, 1988) |
| | | – Storage (2–4 months) | | |
| | Soft cheese | – Melted | ND-tr | |
| | | – Storage (6–18 months) | | |
| | Cheese | – Grated | tr-0.5 μg/g sample | |
| | | – Storage (4–12 months) | | |
| | Dairy spreads | Storage (13 week) at 20 °C | 1.3 μg/g sample | (Nielsen et al., 1996a) |
| | Feta cheese | – Produced from butter oil | 0.3 μg/g sample | (Nielsen et al., 1996b) |
| | | – Storage (5 months) at 4 °C | | |
| | Yellow cheese | – Sliced, fluorescent light | 0.1 μg/g sample | (Nielsen et al., 1995) |
| | | – Storage (55 days) at 4 °C | | |

(*continued on next page*)

*Figure 6:* *(cont'd)*



**Table 4** (*continued*)

| COPs detected | Matrix | Process parameters | Quantity | Reference |
|---|---|---|---|---|
| **5,6β-epoxy** | Infant formula | N/A | 4–46 μg/g sample | (Guardiola et al., 2002) |
| | Milk powder | – Skimmed milk IF powder (18 months old and opened, A)<br>– Skimmed milk IF powder (B)<br>– Skimmed milk powder (C)<br>– Instant formula for ginseng coffee (D)<br>– Instant formula for cream coffee (E) | – 967 ± 59 ng/g sample (A)<br>– 66 ± 20 ng/g sample (B)<br>– 123 ± 17 ng/g sample (C)<br>– 51 ± 9 ng/g sample (D)<br>– 146 ± 6 ng/g sample (E) | (Gorassini et al., 2017) |
| | Butter | – Storage<br>• Fresh<br>• 3–6 months (unheated)<br>• 3–6 months (after heating for 10–20 min at 170–180 °C) | – 4.3–7.3 μg/g sample (fresh)<br>– ND-1.5 μg/g sample (3–6 months, unheated)<br>– 4.3–18.4 μg/g sample (3–6 months, after heating for 10–20 min at 170–180 °C) | (Pie et al., 1991) |
| | Powdered cheese | – Storage:<br>• 6 months at 21 and 38 °C<br>• 18 months at 4 °C | – ND-3 μg/g sample (6 months at 21 and 38 °C)<br>– ND-13 μg/g sample (18 months at 4 °C) | (Sander et al., 1989a,b) |
| | Cheese spread | N/A | 0.71 ±0.03 μg/g sample | (Schmarr et al., 1996) |
| | Parmesan cheese | N/A | 0.71 ±0.21 μg/g sample | |
| | Hard cheese | – Melted<br>– Storage (2–4 months) | tr | (Nourooz-Zadeh and Appelqvist, 1988) |
| | Cheese | – Grated<br>– Storage (4–12 months) | ND-0.3 μg/g sample | |
| | Dairy spreads | Storage (13 week) at 20 °C | 0.67 μg/g sample | (Nielsen et al., 1996a) |
| | Feta cheese | – Produced from butter oil<br>– Storage (5 months) at 4 °C | 0.1 μg/g sample | (Nielsen et al., 1996b) |
| | Yellow cheese | – Sliced, fluorescent light<br>– Storage (55 days) at 4 °C | 0.2 μg/g sample | (Nielsen et al., 1995) |
| **Triol** | Infant formula | Fresh | < 0.01 μg/g sample | (Rose-Sallin et al., 1995) |
| | | N/A | ND-35 μg/g sample | (Guardiola et al., 2002) |
| | Milk powder | Full cream (stored for 3–12 months) | < 0.01–0.063 μg/g sample | (Rose-Sallin et al., 1995) |
| | | Whole milk (fresh) | 0.1 ± 0.0 μg/g sample (fresh) | (Angulo et al., 1997) |
| | | – Skimmed milk infant formula powder (18 months old and opened, A)<br>– Skimmed milk infant formula powder (B)<br>– Skimmed milk powder (C)<br>– Instant formula for ginseng coffee (D)<br>– Instant formula for cream coffee (E) | – 273 ± 24 ng/g sample (A)<br>– 44 ± 9 ng/g sample (B)<br>– 56 ± 20 ng/g sample (C)<br>– 33 ± 4 ng/g sample (D)<br>– 64 ± 10 ng/g sample (E) | (Gorassini et al., 2017) |
| | | For unweaned baby | 6.0 and 3.1 ng/mL sample | (Calderón-Santiago et al., 2012) |
| | UHT milk | – Full-cream<br>– Semi-skimmed | – 1.1 ng/mL sample (full-cream)<br>– 0.7 ng/mL sample (semi-skimmed) | |
| | Butter | – Storage<br>• fresh<br>• 3–6 months (after heating for 10–20 min at 170–180 °C) | – tr (fresh)<br>– tr-0.4 μg/g sample (3–6 months, after heating for 10–20 min at 170–180 °C) | (Pie et al., 1991) |
| | Powdered cheese | – Storage:<br>• 6 months at 21 and 38 °C<br>• 18 months at 4 °C | – ND-3 μg/g sample (6 months at 21 and 38 °C)<br>– ND-17 μg/g sample (18 months at 4 °C) | (Sander et al., 1989a,b) |
| | Dairy spreads | Storage (13 week) at 20 °C | 0.96 μg/g sample | (Nielsen et al., 1996a) |
| | Feta cheese | – Produced from butter oil<br>– Storage (5 months) at 4 °C | 0.3 μg/g sample | (Nielsen et al., 1996b) |

(*continued on next page*)

*Figure 6:* *(cont'd)*



**Table 4** (*continued*)

| COPs detected | Matrix | Process parameters | Quantity | Reference |
|---|---|---|---|---|
| **25-OH** | Infant formula | Fresh | < 0.1 μg/g sample | (Rose-Sallin et al., 1995) |
| | | N/A | ND-19 μg/g sample | (Guardiola, 1994; Guardiola et al., 2002) |
| | | – Sample A | – 0.39 ± 0.03 μg/g lipid extract (Sample A) | (Przygonski et al., 2000) |
| | | – Sample B | – 0.43 ± 0.08 μg/g lipid extract (Sample B) | |
| | | – Sample C | – 0.42 ± 0.05 μg/g lipid extract (Sample C) | |
| | | – Sample D | – 0.55 ± 0.24 μg/g lipid extract (Sample D) | |
| | | – Sample E | – 1.47 ± 0.16 μg/g lipid extract (Sample E) | |
| | Milk powder | Full cream (stored for 3–12 months) | < 0.1–0.132 μg/g sample | (Rose-Sallin et al., 1995) |
| | | – Sample F | – 0.64 ± 0.16 μg/g lipid extract (Sample F) | (Przygonski et al., 2000) |
| | | – Sample G | – 0.64 ± 0.29 μg/g lipid extract (Sample G) | |
| | | – Sample H | – 0.50 ± 0.02 μg/g lipid extract (Sample H) | |
| | | Artificial light in the presence of air for 19 days | 0.375 ± 0.106 μg/g sample | (Dionisi et al., 1998) |
| | | Skimmed | 5.1 ng/mL sample (skimmed) | (Calderón-Santiago et al., 2012) |
| | UHT milk | Skimmed | 3.3 ng/mL sample (skimmed) | |
| | Butter | – Storage<br>• fresh<br>• 3–6 months (after heating for 10–20 min at 170–180 °C) | – tr (fresh)<br>– tr-0.6 μg/g sample (3–6 months, after heating for 10–20 min at 170–180 °C) | (Pie et al., 1991) |
| | Powdered cheese | – Storage:<br>• 6 months at 21 and 38 °C<br>• 18 months at 4 °C | – ND-6 μg/g sample (6 months at 21 and 38 °C)<br>– ND-4 μg/g sample (18 months at 4 °C) | (Sander et al., 1989a,b) |
| | Cheese spread | N/A | 0.51 ± 0.02 μg/g sample | (Schmarr et al., 1996) |
| | Parmesan cheese | N/A | 0.57 ± 0.05 μg/g sample | |
| **20-OH** | Milk powder | – Sample G | – 0.09 ± 0.07 μg/g lipid extract (Sample G) | (Przygonski et al., 2000) |
| | | – Sample H | – 0.04 ± 0.03 μg/g lipid extract (Sample H) | |
| **Total COPs** | Butter oil | N/A | 27.3 μg/g | (Brzeska et al., 2016) |
| | Fruity yogurts | N/A | 13.0 to 18.1 μg/g fat | |
| **Mixture** (7α-OH. 7β-OH, 5,6β-epoxy, Triol, 25-OH, 7-keto, and unidentified COPs) | Indian sweets (containing Ghee)<br>Butter Oil (ghee, sample 16) | N/A | 1.4–51.2 μg/g lipid | (Ubhayasekera et al., 2006) |
| **Mixture** (7β-OH, 7α-OH, 5,6α-epoxy, Triol, 6-keto, 7-keto, 25-OH) | WMP | – Heating (32 °C and 55 °C)<br>– Storage (12 months) at 55 °C in dark conditions<br>– Air exposure | – WMP 1: 1.1 μg/g<br>– WMP 2: 6.8 μg/g | (Angulo et al., 1997) |
| | SMP | – Heating (32 °C and 55 °C)<br>– Storage (12 months) at 35 °C in dark conditions<br>– Air exposure | – SMP1: 0.1 μg/g<br>– SMP2: 7.7 μg/g | |
| | ICP | – Heating (32 °C and 55 °C)<br>– Storage (12 months)<br>– Air exposure | 6.2 μg/g | |
| | ILP | – Heating (32 °C and 55 °C)<br>– Storage (12 months)<br>– Air exposure | 9.2 μg/g | |

**Abbreviations**: tr = trace, N/A = not applicable, ND = Not Determined, NS = Not Specified, IF = Infant Formula, CHOL = Cholesterol, WMP = Whole Milk Powder, SMP = Skim Milk Powder, ICP = Isolate Casein Powder, ILP = Isolated lactoalbumin powder, HTD = High temperature drying.

*Figure 6:* *(cont'd)*



**Table 5**
COPs content in eggs and eggs products.

| COPs detected | Matrix | Process parameters | Quantity | Reference |
|---|---|---|---|---|
| **7α-OH** | Boiled egg | – Heating for 3 and 10 minutes | – 8 μg/g sample (3 minute)<br>– 4 μg/g sample (10 minutes) | (Sarantinos, 1993) |
| | Tea-Leaf egg | – Heating time (0–48 hours)<br>– Ingredients in marinated juice (100% water, 1% soy sauce, 1% tea leaf, 10% soy sauce, 10% tea leaf, standard formula) | – 453.6 ± 1.2 ng/g sample (12 hours)<br>– 425.3 ± 3.5 ng/ g sample (100% water) | (Chen et al., 2010) |
| **7β-OH** | Commercial pasteurized liquid eggs | N/A | 1.31–1.62 μg/g sample | (Guardiola, 1994) |
| | Fried egg | Heating for 1 and 2 minutes | – 34 μg/g sample (1 minute)<br>– 57 μg/g sample (2 minutes) | (Sarantinos, 1993) |
| | Boiled egg | Heating for 3 and 10 minutes | – 38 μg/g sample (3 minute)<br>– 59 μg/g sample (10 minutes) | |
| | Freeze-dried egg | N/A | ND-1.21 μg/g sample | (Guardiola, 1994; Tsai and Hudson, 1984) |
| | Egg pasta | – Treatment A (no 2nd treatment)<br>– treatment B (45 min @ 100 °C)<br>– Treatment C (85 min @ 100 °C)<br>– Heat treatment D (140 min @ 100 °C) | – 17.80 ± 2.3 μg/g cholesterol (A)<br>– 19.44 ± 5.3 μg/g cholesterol (B)<br>– 26.53 ± 5.2 μg/g cholesterol (C)<br>– 27.98 ± 4.2 μg/g cholesterol (D) | (Zardetto et al., 2014) |
| | Tea-Leaf egg | – Heating time (0–48 hours)<br>– Ingredients in marinated juice (100% water, 1% soy sauce, 1% tea leaf, 10% soy sauce, 10% tea leaf, standard formula) | – 539.2 ± 16.2 ng/g sample (12 hours)<br>– 484.4 ± 18.2 ng/ g sample (100% water) | (Chen et al., 2010) |
| **7-keto** | Commercial pasteurized liquid eggs | N/A | 1.12–1.36 μg/g sample | (Guardiola, 1994) |
| | Freeze-dried egg | N/A | ND-1.03 μg/g sample | (Guardiola, 1994; Tsai and Hudson, 1984) |
| | Egg pasta | N/A<br>– Heat treatment A (no 2nd treatment)<br>– Heat treatment B (45 min @ 100 °C)<br>– Heat treatment C (85 min @ 100 °C)<br>– Heat treatment D (140 min @ 100 °C) | 0.1–2.3 μg/g sample<br>– 28.81 ± 4.5 μg/g cholesterol (A)<br>– 37.83 ± 9.3 μg/g cholesterol (B)<br>– 48.84 ± 4.1 μg/g cholesterol (C)<br>– 55.93 ± 6.8 μg/g cholesterol (D) | (Guardiola et al., 2002)<br>(Zardetto et al., 2014) |
| | Tea-Leaf egg | – Heating time (0–48 hours)<br>– Ingredients in marinated juice (100% water, 1% soy sauce, 1% tea leaf, 10% soy sauce, 10% tea leaf, standard formula) | – 316.0 ± 1.4 ng/g sample (24 hours)<br>– 247.8 ± 1.1 ng/ g sample (100% water) | (Chen et al., 2010) |
| **5,6α-epoxy** | Commercial pasteurized liquid eggs | N/A | 0.36–0.45 μg/g sample | (Guardiola, 1994) |
| | Fried egg | Heating 2 minutes | 22 μg/g sample | (Sarantinos, 1993) |
| | Boiled egg | Heating for 3 and 10 minutes | – 28 μg/g sample (3 minute)<br>– 65 μg/g sample (10 minutes) | |
| | Freeze-dried egg | N/A | ND-0.24 μg/g sample | (Guardiola, 1994; Tsai and Hudson, 1984) |
| | Egg pasta | – Heat treatment A (no 2nd treatment)<br>– Heat treatment B (45 min @ 100 °C)<br>– Heat treatment C (85 min @ 100 °C)<br>– Heat treatment D (140 min @ 100 °C) | – 3.73 ± 0.5 μg/g cholesterol (A)<br>– 4.9 ± 1.8 μg/g cholesterol (B)<br>– 5.75 ± 0.5 μg/g cholesterol (C)<br>– 7.08 ± 0.6 μg/g cholesterol (D) | (Zardetto et al., 2014) |

(*continued on next page*)

*Figure 6:* *(cont'd)*



**Table 5** (*continued*)

| COPs detected | Matrix | Process parameters | Quantity | Reference |
|---|---|---|---|---|
| **5,6β-epoxy** | Egg pasta | – Heat treatment A (no 2nd treatment)<br>– Heat treatment B (45 min @ 100 °C)<br>– Heat treatment C (85 min @ 100 °C)<br>– Heat treatment D (140 min @ 100 °C) | – 10.76 ± 2.9 μg/g cholesterol (A)<br>– 11.72 ± 2.0 μg/g cholesterol (B)<br>– 14.81 ± 1.0 μg/g cholesterol (C)<br>– 20.83 ± 1.5 μg/g cholesterol (D) | (Zardetto et al., 2014) |
| | Tea-Leaf egg | – Heating time (0–48 hours)<br>– Ingredients in marinated juice (100% water, 1% soy sauce, 1% tea leaf, 10% soy sauce, 10% tea leaf, standard formula) | – 809.2 ± 14.2 ng/g sample (24 hours)<br>– 811.3 ± 13.3 ng/ g sample (100% water) | (Chen et al., 2010) |
| **25-OH** | Egg pasta | – Heat treatment A (no 2nd treatment)<br>– Heat treatment B (45 min @ 100 °C)<br>– Heat treatment C (85 min @ 100 °C)<br>– Heat treatment D (140 min @ 100 °C) | – 9.14 ± 3.6 μg/g cholesterol (A)<br>– 6.26 ± 2.2 μg/g cholesterol (B)<br>– 7.68 ± 5.8 μg/g cholesterol (C)<br>– 9.39 ± 2.8 μg/g cholesterol (D) | (Zardetto et al., 2014) |
| **Triol** | Commercial pasteurized liquid eggs | N/A | 0.25–0.37 μg/g sample | (Guardiola, 1994) |
| | Fried egg | – Heating for 1 and 2 minutes | – 2 μg/g sample (1 minute)<br>– 3 μg/g sample (2 minutes) | (Sarantinos, 1993) |
| | Boiled egg | – Heating for 3 and 10 minutes | – 1 μg/g sample (3 minute)<br>– 8 μg/g sample (10 minutes) | |
| | Tea-Leaf egg | – Heating time (0–48 hours)<br>– Ingredients in marinated juice (100% water, 1% soy sauce, 1% tea leaf, 10% soy sauce, 10% tea leaf, standard formula) | – 220.3 ± 0.3 ng/g sample (24 hours)<br>– 211.1 ± 1.2 ng/ g sample (1% soy sauce) | (Chen et al., 2010) |
| **Mixture** (7α-OH, 7β-OH, 25-OH, 5,6α-epoxy, 5,6β-epoxy, 6-Keto, 7-keto) | Egg yolk powder | – Radiation absorbed dose: 0, 2.5 or 5 kGy<br>– Vacuum packed<br>– Storage (90 days) at 22 °C. Dark conditions and 73% humidity. | 467 μg/g | (Du and Ahn, 2000) |
| **Mixture** (7β-OH, 5,6β-OH, 5,6α-OH, 25-OH, 7-keto) | Fresh egg pasta | – Variable heating times (1.5,45, 85, 120 min) and temperatures:<br>● group A: 96 °C<br>● group B: 100 °C<br>● group C: 100 °C | – Total COP in μg/g cholesterol:<br>● group A: 71.7<br>● group B: 78.12<br>● group C: 103.7<br>● group D: 117.9 | (Zardetto et al., 2014) |
| **Mixture** (7α-OH, 7β-OH, 5,6β-epoxy, 5,6α-epoxy, 7-keto) | Pasta made from egg products | – Heating (for 6 hours and 20 hours) at LTD and HTD | ● HTD: 95.5 μg/g fat<br>● LTD: 43. 8 μg/g fat | (Verardo et al., 2016) |

**Abbreviations**: tr = trace, N/A = not applicable, ND = Not Determined, NS = Not Specified, LTD = Low temperature drying, HTD = High temperature drying.

*Figure 6:* *(cont'd)*



**Table 6**
COPs content in seafood and seafood products.

| COPs detected | Type of seafood | Process parameters | Quantity | Reference |
|---|---|---|---|---|
| **7-keto** | Anchovy (*Engraulis japonicas*) | Salted-dried | 46.4 μg/g seafood (dry weight basis) | (Ohshima et al., 1993) |
| | Northern cod (*Eleginus gracilis*) | Salted-dried | 9.7 μg/g seafood (dry weight basis) | |
| | | – Raw | – 9.8 μg/g seafood (dry weight basis) – raw | (Li et al., 1994) |
| | | – Grilled | – 8.6 μg/g seafood (dry weight basis) - grilled | |
| | Pacific cod (*Gadus microcephalus*) | Salted-dried | 3.8 μg/g seafood (dry weight basis) | (Ohshima et al., 1993) |
| | | – Raw | – 4.0 μg/g seafood (dry weight basis) - raw | (Shozen et al., 1995) |
| | | – Grilled | – 2.3 μg/g seafood (dry weight basis) - grilled | |
| | Japanese whiting (*Sillago japonica*) | Salted-dried | 24.9 μg/g seafood (dry weight basis) | (Ohshima et al., 1993) |
| | | – Raw | – 24.9 μg/g seafood (dry weight basis) – raw | (Shozen et al., 1995) |
| | | – Grilled | – 36.9 μg/g seafood (dry weight basis) - grilled | |
| | Pacific saury (*Cololabis saira*) | Salted-dried | 7.8 μg/g seafood (dry weight basis) | (Ohshima et al., 1993) |
| | Pacific herring (*Clupea pallasii*) | Salted-dried | 7.5 μg/g seafood (dry weight basis) | |
| | | – Raw | – 46.4 μg/g seafood (dry weight basis) – raw | (Shozen et al., 1995) |
| | | – Grilled | – 53.3 μg/g seafood (dry weight basis) - grilled | |
| | Anchovy (*E. japonicas*) | Boiled-dried | 60.6 μg/g seafood (dry weight basis) | (Ohshima et al., 1993) |
| | Shrimp (*Sergestes lucens*) | Boiled-dried | 4.0 μg/g seafood (dry weight basis) | |
| | Anchovy (*E. japonicas*) | Boiled-dried | 40.7 μg/g seafood (dry weight basis) | |
| | Keta salmon (*Onchorhynchus keta*) | Smoked | 6.3 μg/g seafood (dry weight basis) | |
| | Squid buccal mass | – Raw | – 7.9 μg/g seafood (dry weight basis) – raw | (Shozen et al., 1995) |
| | | – Grilled | – 8.3 μg/g seafood (dry weight basis) - grilled | |
| | Horse mackerel | – Raw | – 11.1 μg/g seafood (dry weight basis) – raw | |
| | | – Grilled | – 7.8 μg/g seafood (dry weight basis) - grilled | |
| | Sardine | Air-dried | 5.3 mg/100 g wet basis | (Osada et al., 1993) |
| | Squid | Air-dried | 1.9 mg/100 g wet basis | (Osada et al., 1993) |
| | Tuna | Boiled-canned | 37.4 μg/g lipid | (Zunin et al., 2001) |
| | Alaskan pollack roe | Pickled and spiced | 3.3 mg/100 g sample | (Osada et al., 1993) |
| | Shrimp | – Raw | – 7.5 ± 4.3 μg/g lipid (raw) | (Hernandez Becerra et al., 2014) |
| | | – Boiled (washed with a 5% NaCl solution and dried under direct sunlight for periods of 8 hours per day for 4 days) | – 6.8 ± 5.4 μg/g lipid (boiled) | |
| | | – Dried and stored (0–90 days) | – 52.9 ± 5.8 (0 days) | |
| | | | – 213.6 ± 16.9 (90 days) | |
| | Fish patés | – Salmon (cooked) | – 35 ± 5 μg/100 g product (salmon) | (Echarte et al., 2004) |
| | | – Cod (cooked) | – 67 ± 4 μg/100 g product (cod) | |
| **7α-OH** | Sardine | Air-dried | 2.7 mg/100 g wet basis | (Osada et al., 1993) |
| | Tuna | Boiled-canned | 17.3 μg/g lipid | (Zunin et al., 2001) |
| | Alaskan pollack roe | Pickled and spiced | 3.8 mg/100 g sample | (Osada et al., 1993) |
| | Shrimp | – Raw | – 5.8 ± 2.6 μg/g lipid (raw) | (Hernandez Becerra et al., 2014) |
| | | – Boiled (washed with a 5% NaCl solution and dried under direct sunlight for periods of 8 hours per day for 4 days) | – 7.8 ± 3.5 μg/g lipid (boiled) | |
| | | – Dried and stored (0–90 days) | – 90.3 ± 16.5 (0 days) | |
| | | | – 135.6 ± 21.1 (90 days) | |
| | Fish patés | – Salmon (cooked) | – 53 ± 7 μg/100 g product (salmon) | (Echarte et al., 2004) |
| | | – Anchovy (cooked) | – 33 ± 3 μg/100 g product (anchovy) | |
| | | – Cod (cooked) | | |

(*continued on next page*)

*Figure 6:* *(cont'd)*



**Table 6** (*continued*)

| COPs detected | Type of seafood | Process parameters | Quantity | Reference |
|---|---|---|---|---|
| | | | – 71 ± 1 μg/100 g product (cod) | |
| **7β-OH** | Anchovy (*Engraulis japonicas*) | Salted-dried | 37.1 μg/g seafood (dry weight basis) | (Ohshima et al., 1993) |
| | Northern cod (*Eleginus gracilis*) | Salted-dried | 6.8 μg/g seafood (dry weight basis) | |
| | | – Raw | – 6.8 μg/g seafood (dry weight basis) – raw | (Shozen et al., 1995) |
| | | – Grilled | – 12.4 μg/g seafood (dry weight basis) - grilled | |
| | Pacific cod (*Gadus microcephalus*) | Salted-dried | 3.7 μg/g seafood (dry weight basis) | (Ohshima et al., 1993) |
| | | – Raw | – 4.0 μg/g seafood (dry weight basis) – raw | (Shozen et al., 1995) |
| | | – Grilled | – 5.6 μg/g seafood (dry weight basis) - grilled | |
| | Japanese whiting (*Sillago japonica*) | Salted-dried | 24.5 μg/g seafood (dry weight basis) | (Ohshima et al., 1993) |
| | | – Raw | – 24.5 μg/g seafood (dry weight basis) – raw | (Shozen et al., 1995) |
| | | – Grilled | – 32.8 μg/g seafood (dry weight basis) - grilled | |
| | Pacific saury (*Cololabis saira*) | Salted-dried | 9.9 μg/g seafood (dry weight basis) | (Ohshima et al., 1993) |
| | Pacific herring (*Clupea pallasii*) | Salted-dried | 8.4 μg/g seafood (dry weight basis) | |
| | | – Raw | – 37.1 μg/g seafood (dry weight basis) – raw | (Shozen et al., 1995) |
| | | – Grilled | – 46.4 μg/g seafood (dry weight basis) - grilled | |
| | Anchovy (*E. japonicas*) | Boiled-dried | 55.8 μg/g seafood (dry weight basis) | (Ohshima et al., 1993) |
| | Shrimp (*Sergestes lucens*) | Boiled-dried | 3.7 μg/g seafood (dry weight basis) | |
| | Anchovy (*E. japonicas*) | Boiled-dried | 43.8 μg/g seafood (dry weight basis) | |
| | Keta salmon (*Onchorhynchus keta*) | Smoked | 7.3 μg/g seafood (dry weight basis) | |
| | Squid buccal mass | – Raw | – 15.2 μg/g seafood (dry weight basis) – raw | (Shozen et al., 1995) |
| | | – Grilled | – 18.3 μg/g seafood (dry weight basis) - grilled | |
| | Horse mackerel | – Raw | – 12.1 μg/g seafood (dry weight basis) – raw | |
| | | – Grilled | – 9.5 μg/g seafood (dry weight basis) - grilled | |
| | Sardine | – Air-dried | – 9.8 mg/100 g wet basis | (Osada et al., 1993) |
| | | – Boiled-canned | – 2.8 mg/100 g wet basis | |
| | Squid | Air-dried | 5.5 mg/100 g wet basis | (Osada et al., 1993) |
| | Tuna | Boiled-canned | 21.5 μg/g lipid | (Zunin et al., 2001) |
| | Alaskan pollack roe | Pickled and spiced | 5.8 mg/100 g sample | (Osada et al., 1993) |
| | Shrimp | – Raw | – 4.7 ± 3.1 μg/g lipid (raw) | (Hernandez Becerra et al., 2014) |
| | | – Boiled (washed with a 5% NaCl solution and dried under direct sunlight for periods of 8 hours per day for 4 days) | – 5.8 ± 4.6 μg/g lipid (boiled) | |
| | | | – 91.0 ± 7.8 (0 days) | |
| | | – Dried and stored (0–90 days) | – 221.9 ± 15.9 (90 days) | |
| | Fish patés | – Salmon (cooked) | – 46 ± 5 μg/100 g product (salmon) | (Echarte et al., 2004) |
| | | – Anchovy (cooked) | – 27 ± 1 μg/100 g product (anchovy) | |
| | | – Cod (cooked) | – 74 ± 3 μg/100 g product (cod) | |
| **5,6α-epoxy** | Anchovy (*Engraulis japonicas*) | Salted-dried | 11.9 μg/g seafood (dry weight basis) | (Ohshima et al., 1993) |
| | Northern cod (*Eleginus gracilis*) | Salted-dried | 2.9 μg/g seafood (dry weight basis) | |
| | | – Raw | – 2.9 μg/g seafood (dry weight basis) – raw | (Shozen et al., 1995) |
| | | – Grilled | – 2.8 μg/g seafood (dry weight basis) - grilled | |
| | Pacific cod (*Gadus microcephalus*) | Salted-dried | 3.1 μg/g seafood (dry weight basis) | (Ohshima et al., 1993) |

(*continued on next page*)

*Figure 6:* *(cont'd)*



**Table 6** (*continued*)

| COPs detected | Type of seafood | Process parameters | Quantity | Reference |
|---|---|---|---|---|
| | | – Raw<br>– Grilled | – 3.4 μg/g seafood (dry weight basis) – raw<br>– 2.9 μg/g seafood (dry weight basis) - grilled | (Shozen et al., 1995) |
| | Japanese whiting (*Sillago japonica*) | Salted-dried<br>– Raw<br>– Grilled | 8.9 μg/g seafood (dry weight basis)<br>– 8.9 μg/g seafood (dry weight basis) – raw<br>– 12.7 μg/g seafood (dry weight basis) - grilled | (Ohshima et al., 1993)<br>(Shozen et al., 1995) |
| | Pacific saury (*Cololabis saira*) | Salted-dried | tr | (Ohshima et al., 1993) |
| | Pacific herring (*Clupea pallasii*) | Salted-dried<br>–<br>– Raw<br>– Grilled | 3.5 μg/g seafood (dry weight basis)<br>– 11.9 μg/g seafood (dry weight basis) – raw<br>– 11.6 μg/g seafood (dry weight basis) - grilled | (Shozen et al., 1995) |
| | Anchovy (*E. japonicas*) | Boiled-dried | 18.0 μg/g seafood (dry weight basis) | (Ohshima et al., 1993) |
| | Shrimp (*Sergestes lucens*) | Boiled-dried | tr | |
| | Anchovy (*E. japonicas*) | Boiled-dried | 12.5 μg/g seafood (dry weight basis) | |
| | Keta salmon (*Onchorhynchus keta*) | Smoked | 2.4 μg/g seafood (dry weight basis) | |
| | Squid buccal mass | – Raw<br>– Grilled | – 5.9 μg/g seafood (dry weight basis) – raw<br>– 5.0 μg/g seafood (dry weight basis) - grilled | (Shozen et al., 1995) |
| | Horse mackerel | – Raw<br>– Grilled | – 7.2 μg/g seafood (dry weight basis) – raw<br>– 6.6 μg/g seafood (dry weight basis) - grilled | |
| | Sardine | – Air-dried<br>– Boiled-canned | – 1.1 mg/100 g wet basis<br>– 0.2 mg/100 g wet basis | (Osada et al., 1993) |
| | Squid | Air-dried | 1.4 mg/100 g sample | (Osada et al., 1993) |
| | Tuna | Boiled-canned | 9.4 μg/g lipid | (Zunin et al., 2001) |
| | Alaskan pollack roe | Pickled and spiced | 0.8 mg/100 g sample | (Osada et al., 1993) |
| | Shrimp | – Raw<br>– Boiled (washed with a 5% NaCl solution and dried under direct sunlight for periods of 8 hours per day for 4 days)<br>– Dried and stored (0–90 days) | – 12.9 ± 7.4 μg/g lipid (raw)<br>– 14.0 ± 6.9 μg/g lipid (boiled)<br>– 65.1 ± 4.9 (0 days)<br>– 118.5 ± 20.4 (90 days) | (Hernandez Becerra et al., 2014) |
| | Fish patés | Salmon (cooked) | 13 ± 0 μg/100 g product (salmon) | (Echarte et al., 2004) |
| **5,6β-epoxy** | Anchovy (Engraulis japonicas) | Salted-dried | 33.3 μg/g seafood (dry weight basis) | (Ohshima et al., 1993) |
| | Northern cod (Eleginus gracilis) | Salted-dried<br>– Raw<br>– Grilled | 5.5 μg/g seafood (dry weight basis)<br>– 5.4 μg/g seafood (dry weight basis) – raw<br>– 5.1 μg/g seafood (dry weight basis) - grilled | (Shozen et al., 1995) |
| | Pacific cod (Gadus microcephalus) | Salted-dried<br>– Raw<br>– Grilled | 5.9 μg/g seafood (dry weight basis)<br>– 5.9 μg/g seafood (dry weight basis) – raw<br>– 6.2 μg/g seafood (dry weight basis) - grilled | (Ohshima et al., 1993)<br>(Shozen et al., 1995) |
| | Japanese whiting (*Sillago japonica*) | Salted-dried<br>– Raw<br>– Grilled | 16.1 μg/g seafood (dry weight basis)<br>– 16.1 μg/g seafood (dry weight basis) – raw<br>– 25.0 μg/g seafood (dry weight basis) - grilled | (Ohshima et al., 1993)<br>(Shozen et al., 1995) |
| | Pacific saury (*Cololabis saira*) | Salted-dried | tr | (Ohshima et al., 1993) |
| | Pacific herring (*Clupea pallasii*) | Salted-dried | 5.4 μg/g seafood (dry weight basis) | |

(*continued on next page*)

*Figure 6:* *(cont'd)*



**Table 6** (*continued*)

| COPs detected | Type of seafood | Process parameters | Quantity | Reference |
|---|---|---|---|---|
| | | – Raw<br>– Grilled | – 33.4 μg/g seafood (dry weight basis) – raw<br>– 33.4 μg/g seafood (dry weight basis) - grilled | (Shozen et al., 1995) |
| | Anchovy (*E. japonicas*) | Boiled-dried | 43.3 μg/g seafood (dry weight basis) | (Ohshima et al., 1993) |
| | Shrimp (*Sergestes lucens*) | Boiled-dried | tr | |
| | Anchovy (*E. japonicas*) | Boiled-dried | 26.6 μg/g seafood (dry weight basis) | |
| | Keta salmon (Onchorhynchus keta) | Smoked | 3.3 μg/g seafood (dry weight basis) | |
| | Squid buccal mass | – Raw<br>– Grilled | – 10.8 μg/g seafood (dry weight basis) – raw<br>– 10.0 μg/g seafood (dry weight basis) - grilled | (Shozen et al., 1995) |
| | Horse mackerel | – Raw<br>– Grilled | – 10.6 μg/g seafood (dry weight basis) – raw<br>– 8.6 μg/g seafood (dry weight basis) - grilled | |
| | Sardine | – Air-dried<br>– Boiled-canned | – 4.9 mg/100 g wet basis<br>– 0.7 mg/100 g wet basis | (Osada et al., 1993) |
| | Squid | Air-dried | 2.2 mg/100 g sample | (Osada et al., 1993) |
| | Tuna | Boiled-canned | 18.5 μg/g lipid | (Zunin et al., 2001) |
| | Alaskan pollack roe | Pickled and spiced | 1.0 mg/100 g sample | (Osada et al., 1993) |
| | Shrimp | – Raw<br>– Boiled (washed with a 5% NaCl solution and dried under direct sunlight for periods of 8 hours per day for 4 days)<br>– Dried and stored (0–90 days) | – 4.7 ± 2.1 μg/g lipid (raw)<br>– 4.4 ± 2.7 μg/g lipid (boiled)<br>– 55.0 ± 1.8 (0 days)<br>– 145.3 ± 18.9 (90 days) | (Hernandez Becerra et al., 2014) |
| **Triol** | Anchovy (*Engraulis japonicas*) | Salted-dried | 3.5 μg/g seafood (dry weight basis) | (Ohshima et al., 1993) |
| | Northern cod (*Eleginus gracilis*) | Salted-dried | 1.5 μg/g seafood (dry weight basis) | |
| | | – Raw<br>– Grilled | – 1.5 μg/g seafood (dry weight basis) – raw<br>– 2.4 μg/g seafood (dry weight basis) - grilled | (Shozen et al., 1995) |
| | Pacific cod (*Gadus microcephalus*) | Salted-dried | 1.8 μg/g seafood (dry weight basis) | (Ohshima et al., 1993) |
| | | – Raw<br>– Grilled | – 1.9 μg/g seafood (dry weight basis) – raw<br>– 1.1 μg/g seafood (dry weight basis) - grilled | (Shozen et al., 1995) |
| | Japanese whiting (*Sillago japonica*) | Salted-dried | 3.4 μg/g seafood (dry weight basis) | (Ohshima et al., 1993) |
| | | – Raw<br>– Grilled | – 3.5 μg/g seafood (dry weight basis) – raw<br>– 1.3 μg/g seafood (dry weight basis) - grilled | (Shozen et al., 1995) |
| | Pacific saury (*Cololabis saira*) | Salted-dried | tr | (Ohshima et al., 1993) |
| | Pacific herring (*Clupea pallasii*) | Salted-dried | 3.5 μg/g seafood (dry weight basis) | |
| | | – Raw<br>– Grilled | – 3.5 μg/g seafood (dry weight basis) – raw<br>– 4.5 μg/g seafood (dry weight basis) - grilled | (Shozen et al., 1995) |
| | Anchovy (*E. japonicas*) | Boiled-dried | 8.5 μg/g seafood (dry weight basis) | (Ohshima et al., 1993) |
| | Shrimp (*Sergestes lucens*) | Boiled-dried | tr | |
| | Anchovy (*E. japonicas*) | Boiled-dried | 39.1 μg/g seafood (dry weight basis) | |
| | Keta salmon (*Onchorhynchus keta*) | Smoked | 2.7 μg/g seafood (dry weight basis) | |
| | Squid buccal mass | – Raw<br>– Grilled | – 4.8 μg/g seafood (dry weight basis) – raw<br>– 3.9 μg/g seafood (dry weight basis) - grilled | (Shozen et al., 1995) |

(*continued on next page*)

*Figure 6:* *(cont'd)*



**Table 6** (*continued*)

| COPs detected | Type of seafood | Process parameters | Quantity | Reference |
|---|---|---|---|---|
| | Horse mackerel | – Raw<br>– Grilled | – 2.7 μg/g seafood (dry weight basis) – raw<br>– 1.7 μg/g seafood (dry weight basis) - grilled | |
| | Tuna | Boiled-canned | 14.3 μg/g lipid | (Zunin et al., 2001) |
| | Alaskan pollack roe | Pickled and spiced | 0.3 mg/100 g sample | (Osada et al., 1993) |
| | Shrimp | – Dried and stored (0–90 days) | – 2.0 ± 3.5 (0 days)<br>– 21.6 ± 0.4 (90 days) | (Hernandez Becerra et al., 2014) |
| | Fish patés | – Salmon (cooked)<br>– Anchovy (cooked)<br>– Cod (cooked) | – 27 ± 4 μg/100 g product (salmon)<br>– 12 ± 0 μg/100 g product (anchovy)<br>– 23 ± 2 μg/100 g product (cod) | (Echarte et al., 2004) |
| **25-OH** | Anchovy (*Engraulis japonicas*) | Salted-dried | 5.8 μg/g seafood (dry weight basis) | (Ohshima et al., 1993) |
| | Northern cod (*Eleginus gracilis*) | Salted-dried<br>Raw | 0.9 μg/g seafood (dry weight basis)<br>0.9 μg/g seafood (dry weight basis) – raw | (Shozen et al., 1995) |
| | Pacific cod (*Gadus microcephalus*) | Salted-dried<br>– Raw<br>– Grilled | 2.5 μg/g seafood (dry weight basis)<br>– 2.8 μg/g seafood (dry weight basis) – raw<br>– 1.3 μg/g seafood (dry weight basis) - grilled | (Ohshima et al., 1993)<br>(Shozen et al., 1995) |
| | Japanese whiting (*Sillago japonica*) | Salted-dried<br>– Raw<br>– Grilled | 10.7 μg/g seafood (dry weight basis)<br>– 10.8 μg/g seafood (dry weight basis) – raw<br>– 5.1 μg/g seafood (dry weight basis) - grilled | (Ohshima et al., 1993)<br>(Shozen et al., 1995) |
| | Pacific saury (*Cololabis saira*) | Salted-dried | tr | (Ohshima et al., 1993) |
| | Pacific herring (*Clupea pallasii*) | Salted-dried<br>– Raw<br>– Grilled | 5.6 μg/g seafood (dry weight basis)<br>– 5.9 μg/g seafood (dry weight basis) – raw<br>– 6.7 μg/g seafood (dry weight basis) - grilled | (Shozen et al., 1995) |
| | Anchovy (*E. japonicas*) | Boiled-dried | 1.8 μg/g seafood (dry weight basis) | (Ohshima et al., 1993) |
| | Shrimp (*Sergestes lucens*) | Boiled-dried | 0.6 μg/g seafood (dry weight basis) | |
| | Anchovy (*E. japonicas*) | Boiled-dried | 8.5 μg/g seafood (dry weight basis) | |
| | Keta salmon (*Onchorhynchus keta*) | Smoked | 4.8 μg/g seafood (dry weight basis) | |
| | Squid buccal mass | – Raw<br>– Grilled | – 5.8 μg/g seafood (dry weight basis) – raw<br>– 4.8 μg/g seafood (dry weight basis) - grilled | (Shozen et al., 1995) |
| | Horse mackerel | – Raw<br>– Grilled | – 3.6 μg/g seafood (dry weight basis) – raw<br>– 4.0 μg/g seafood (dry weight basis) - grilled | |
| | Tuna | Boiled-canned | 14.3 μg/g lipid | (Zunin et al., 2001) |
| | Shrimp | – Raw<br>– Boiled (washed with a 5% NaCl solution and dried under direct sunlight for periods of 8 hours per day for 4 days)<br>– Dried and stored (0–90 days) | – 3.3 ± 2.0 μg/g lipid (raw)<br>– 4.9 ± 2.9 μg/g lipid (boiled)<br>– 16.6 ± 6.4 (0 days)<br>– 30.1 ± 4.2 (90 days) | (Hernandez Becerra et al., 2014) |
| **Total COPs** | Salmon | – Raw<br>– Fried with olive oil<br>– Fried with soya oil<br>– Roasted | – 0.7 μg/g fat (raw)<br>– 3.0 μg/g fat (fried with olive oil)<br>– 3.4 μg/g fat (fried with soya oil)<br>– 7.4 μg/g fat (roasted) | (Brzeska et al., 2016) |
| **Mixture** (7α-OH, 7β-OH, 5,6β-epoxy, 5,6α-epoxy, 25-OH, 7-keto) | Shrimp | – Boiling direct sunlight (8 hours a day) for 4 days<br>– Storage (90 days) at 25 °C | 886.6 μg/g lipid at 90 days | (Hernandez Becerra et al., 2014) |

(*continued on next page*)

*Figure 6:* *(cont'd)*



**Table 6** (*continued*)

| COPs detected | Type of seafood | Process parameters | Quantity | Reference |
|---|---|---|---|---|
| **Mixture** (7α-OH, 7β-OH, 7-keto) | Atlantic salmon | – Antioxidant Addition and 26 days storage<br>– Added ingredients (1 and 3 g/kg fish):<br>• Rosemary extract<br>• Oregano extract<br>• Antimicrobial blend of seven herbs<br>– Baking for 20 minutes at 180 °C | 0.6–100 meq. $O_2$ / kg lipid extract | (Tarvainen et al., 2015) |

**Abbreviations**: tr = trace, N/A = not applicable, ND = Not Determined, NS = Not Specified.

*Figure 6:* *(cont'd)*



raw food; thus vacuum storage of cooked meat products has been suggested as an alternative to reduce their formation (Min et al., 2016). Reduction in surface area of food exposed to the atmosphere and the amount of light absorbed by the food, as well as the addition of a surface spray and light reflecting or absorbing packaging can all help reduce the formation of COPs (Khan et al., 2015; Li et al., 1996; Mariutti and Bragagnolo, 2017; Medina-Meza et al., 2014a,b; Min et al., 2015; Overholt et al., 2016; Savage et al., 2002). A decrease in time from farm to table could also significantly decrease their accumulation. Changes in food consumption habits can represent a challenge in terms of establishing exposure to toxic compounds derived from diet. In the United States, the consumption of ready to eat (RTE) and "deli" food products have increased in the last decade. Sandwiches, hot dogs, hamburgers, wrap or "subs" are some of the most RTE popular products. Frozen food products are usually thawed and microwaved before consumption. Heat produced during microwaving of food have shown to accelerate the chemical oxidation (Aziz et al., 2002; Picouet et al., 2007; Savage et al., 2002; Yarmand and Homayouni, 2009), compared to traditional cooking. Home meal preparation may affect not only cholesterol and lipids, but also protein fraction and its derivatives enhancing the probability of the food matrix to undergo several oxidative transformations. Logistics between farmers, processors, shipping companies and retail locations could be an important step in reducing the storage time of animal food products. However, although there is some knowledge on the relationship between packaging and storage conditions, there is still a need of experimental data regarding the effect of storage time and other storage conditions (as well as other food cooking methods and conditions) on COPs content. As previously explained, these thermal changes vary among the food matrix, and the cooking methods and conditions; hence, COPs formation becomes not only directly related to the food matrix composition, but also in the processing method, packing and storage conditions.

## 4. COPs: a food toxicological target?

### *4.1. Fate of the COPs: risk and exposure assessment*

Humans are exposed to a variety of substances from multiples exposure routes (i.e. inhalation, contact, ingestion) and sources (i.e. air, food, soil, water) (Dorne et al., 2009). By definition, risk is the probability of an adverse effect on man occurring as a result of a given exposure to a chemical or mixture, while hazard is the inherent capacity of a chemical or mixture to cause adverse effects in man under conditions of exposure (Hanlon et al., 2016). Food toxicology has emerged as a critical topic for both the scientific community and governments, leading to the formation of different projects, like the Total Diet Study (TDS) led by the Food and Drug Administration (FDA, 2014) in the United States. The project started on 1961 and aims to systematically monitoring several contaminants and nutrients present in the average U.S. diet. Unfortunately, even though special attention has been given to the analysis of the American diet, there is no specific study on the risk exposure of COPs. Currently, there are no federal regulations for food processing and storage conditions taking into account the content of COPs and the consequent human risk exposure, even considering the broad body of evidence demonstrating the direct relationship between COPs and several chronical diseases (Del Rio et al., 2013; Mukhopadhyay et al., 2017). This information is essential to pursue more detailed studies on the relationship between COPs' intake and toxicological effects. The TDS is monitoring about 800 compounds in the US diet during 2014–2017 (FDA, 2014). Selected pesticides, herbicides, radionuclides, nutrients and toxic elements are tracked year by year through 4 market basket of food products. The food database used in the TDS can potentially be a good starting point to perform a systematic monitoring of the occurrence of COPs and to estimate the annual dietary intakes in the U.S. population. Gaining a deep understanding of how and from what sources humans consume cholesterol

*Figure 6:* *(cont'd)*



**Table 7**
COPs content in model systems and other food matrices.

| COPs detected | Matrix | Process parameters | Quantity | Reference |
|---|---|---|---|---|
| **7-keto** | Emulsion system | – Antioxidant addition for 5 days:<br>• 20 μg/ml Chinese bayberry sample<br>• 40 μg/ml Chinese bayberry sample | – Control: 287.8 μg/ml<br>– 20 μg/ml Chinese bayberry sample: 107–229 μg/ml | (Zhang et al., 2017) |
| | Indian ghee | – Frying at 180–200 °C for 1 hour, 3 times over 3 days.<br>– Intermittent heating at 225 °C for 30 minutes and stored for 24 hours (repeated for 3 days) | – 14–84.6 μg/g sample (Frying at 180–200 °C for 1 hour, 3 times over 3 days)<br>– 7.9–47.7 μg/g sample (intermittent heating at 225 °C for 30 minutes and stored for 24 hours (repeated for 3 days) | (Kumar and Singhal, 1992) |
| | Heated tallow | N/A | 1–80 μg/g sample | (Guardiola et al., 2002) |
| | Refined lard | N/A | tr-0.3 μg/g sample | |
| | Lard | N/A | 1.94 ± 0.03 μg/g sample | |
| | Fried potatoes | N/A | 4–18 μg/g sample | |
| | Bakery products | N/A | 0.2–0.9 μg/g sample | |
| | Weaning foods | N/A | ND-7 μg/g sample | |
| | Tallow | N/A | 36.87 ± 8.51 μg/g sample | (Chiu et al., 2018) |
| | Breast milk | N/A | 0.2–1.2 μg/g lipid | (Scopesi et al., 2002) |
| | Adapted formula milk | N/A | 0.3–11.2 μg/g lipid | (Scopesi et al., 2002) |
| **7α-OH** | Heated tallow | N/A | 1–80 μg/g sample | (Guardiola et al., 2002) |
| | Refined lard | N/A | tr-0.3 μg/g sample | |
| | Lard | N/A | 0.742 ± 0.060 μg/g sample | (Chiu et al., 2018) |
| | Tallow | N/A | 8.47 ± 0.37 μg/g sample | (Chiu et al., 2018) |
| | Indian ghee | – Frying at 180–200 °C for 1 hour, 3 times over 3 days.<br>– Intermittent heating at 225 °C for 30 minutes and stored for 24 hours (repeated for 3 days) | – 7.1–33.8 μg/g sample (Frying at 180–200 °C for 1 hour, 3 times over 3 days)<br>– 2.9–22.9 μg/g sample (intermittent heating at 225 °C for 30 minutes and stored for 24 hours (repeated for 3 days) | (Kumar and Singhal, 1992) |
| **7β-OH** | Heated tallow | N/A | 1–55 μg/g sample | (Guardiola et al., 2002) |
| | Fried potatoes | N/A | 1–3 μg/g sample | |
| | Weaning foods | N/A | ND-24 μg/g sample | |
| | Lard | N/A | 0.726 ± 0.061 μg/g sample | (Chiu et al., 2018) |
| | Tallow | N/A | 4.81 ± 1.09 μg/g sample | (Chiu et al., 2018) |
| | Indian ghee | – Frying at 180–200 °C for 1 hour, 3 times over 3 days.<br>– Intermittent heating at 225 °C for 30 minutes and stored for 24 hours (repeated for 3 days) | – 22.1–86.9 μg/g sample (Frying at 180–200 °C for 1 hour, 3 times over 3 days)<br>– 7.8–45.9 μg/g sample (intermittent heating at 225 °C for 30 minutes and stored for 24 hours (repeated for 3 days) | (Kumar and Singhal, 1992) |
| **5,6α-epoxy** | Heated tallow | N/A | 1–28 μg/g sample | (Guardiola et al., 2002) |
| | Refined lard | N/A | 0.2–0.4 μg/g sample | |
| | Fried potatoes | N/A | 1–3 μg/g sample | |
| | Weaning foods | N/A | ND-5 μg/g sample | |
| | Tallow | N/A | 6.04 ± 2.09 μg/g sample | (Chiu et al., 2018) |
| | Indian ghee | – Frying at 180–200 °C for 1 hour, 3 times over 3 days.<br>– Intermittent heating at 225 °C for 30 minutes and stored for 24 hours (repeated for 3 days) | – 23.8–95.6 μg/g sample (Frying at 180–200 °C for 1 hour, 3 times over 3 days)<br>– 13.1–68.8 μg/g sample (intermittent heating at 225 °C for 30 minutes and stored for 24 hours (repeated for 3 days) | (Kumar and Singhal, 1992) |
| **5,6β-epoxy** | Heated tallow | N/A | 1–42 μg/g sample | (Guardiola et al., 2002) |
| | Fried potatoes | N/A | 1–2 μg/g sample | |
| | Weaning foods | N/A | ND-5 μg/g sample | |
| | Tallow | N/A | 11.22 ± 3.38 μg/g sample | (Chiu et al., 2018) |
| | Breast milk | Non-lactating women | 352 μg/dl sample (median of samples) | (Gruenke et al., 1987) |
| | Indian ghee | – Frying at 180–200 °C for 1 hour, 3 times over 3 days.<br>– Intermittent heating at 225 °C for 30 minutes and stored for 24 hours (repeated for 3 days) | – 23.8–95.6 μg/g sample (Frying at 180–200 °C for 1 hour, 3 times over 3 days)<br>– 13.1–68.8 μg/g sample (intermittent heating at 225 °C for 30 minutes and stored for 24 hours (repeated for 3 days) | (Kumar and Singhal, 1992) |
| **25-OH** | Heated tallow | N/A | 1–5 μg/g sample | (Guardiola et al., 2002) |
| | Refined lard | N/A | tr-0.2 μg/g sample | |
| | Fried potatoes | N/A | 2–7 μg/g sample | |

(*continued on next page*)

*Figure 6:* *(cont'd)*



**Table 7** (*continued*)

| COPs detected | Matrix | Process parameters | Quantity | Reference |
|---|---|---|---|---|
| | Weaning foods | N/A | ND-14 μg/g sample | |
| | Indian ghee | – Frying at 180–200 °C for 1 hour, 3 times over 3 days.<br>– Intermittent heating at 225 °C for 30 minutes and stored for 24 hours (repeated for 3 days) | – 3.1–16.2 μg/g sample (Frying at 180–200 °C for 1 hour, 3 times over 3 days)<br>– 2.2–10.8 μg/g sample (intermittent heating at 225 °C for 30 minutes and stored for 24 hours (repeated for 3 days) | (Kumar and Singhal, 1992) |
| **Triol** | Lard | N/A | 0.398 ± 0.030 μg/g sample | (Chiu et al., 2018) |
| | Tallow | N/A | 2.74 ± 2.82 μg/g sample | (Chiu et al., 2018) |
| | Indian ghee | – Frying at 180–200 °C for 1 hour, 3 times over 3 days.<br>– Intermittent heating at 225 °C for 30 minutes and stored for 24 hours (repeated for 3 days) | – tr-7.3 μg/g sample (Frying at 180–200 °C for 1 hour, 3 times over 3 days)<br>– tr-7.6 μg/g sample (intermittent heating at 225 °C for 30 minutes and stored for 24 hours (repeated for 3 days) | (Kumar and Singhal, 1992) |
| **Total COPs** | Butter cookies | N/A | 1.7 to 1.9 μg/g | (Brzeska et al., 2016) |
| | Butter cake | N/A | 3.1 to 4.4 μg/g | |
| | Croissant | N/A | 1.1 μg/g | |
| **Mixture** (7β-OH, 7-keto, 5,6β-epoxy, Triol) | Mixture of sardine oil, TAG and Cholesterol | – Storage (39 days) at 25 °C under dark conditions | 68.3–951 μg/g | (Li et al., 1994) |
| **Mixture** (7α-OH, 7β-OH, 7-keto, 5,6α-epoxy, 5,6β-epoxy) | Fish, flax, sunflower, and palm oils | – Storage (35 days) at 25°C under uniform light<br>– Air exposure<br>– Heating (22 hours) at 110 °C | N/A | (Li et al., 1996) |
| **Mixture** (7β-OH, 5β,6β-epoxy, 5α,6α-epoxy, Triol, 25-OH, 7-keto) | Lard | – Heating (120 minutes) at 150 °C<br>– Thickness of layer: cut into 3 slices (10 mm thick) | 1.5–10.4 μg/g | (Derewiaka and Obiedzinski, 2010) |
| **Mixture** (4α-OH, 4β-OH, 6α-OH, 6β-OH, 7α-OH, 7β-OH, 25-OH, 5,6α Epoxy, 5,6β-Epoxy, Triol, 7-keto) | Cholesterol standard | – Heating (18 minutes) at 120, 150, 180 and 220 °C | Total COP content at 150 °C at 120 minutes: 167.7 (mg/g) | (Derewiaka and Molinska nee Sosinska, 2015) |
| **Mixture** (7α-OH, 7β-OH, 5,6β-epoxy, 5,6α-epoxy) | Cholesterol standards (as model for cooking fish) | – Storage (72 hours)<br>– Heating (7 minutes) at 180 °C<br>– Antioxidant addition: 0.2 mL Maná-cubiu extract (MCE) | • Unheated CHO: 0 μg/g<br>• Heated CHO:238.24 μg/g (72 hours)<br>• Heated CHO: + MCE: 23.96 μg/g (72 hours) | (Barriuso et al., 2016) |

**Abbreviations**: tr = trace, N/A = not applicable, ND = Not Determined, NS = Not Specified, TAG = Triacylglycerol, MCE = Mana-cubiu extract.

*Figure 6:* *(cont'd)*



oxidative products could provide information to help reduce consumption. A decrease in COPs consumption would lead a decrease in the side effects of COPs related chronic diseases.

From studies published decades ago, the toxicity and the potential hazard activity of COPs have been demonstrated; however, being an "ensemble" of molecules rather than a single compound, performing an assessment of COPs exposure is challenging. Ideally, occurrence data with concentrations and frequency should be available in exhaustive, consistent lists; in practice, these conditions rarely met. The most common information on food consumption is derived from dietary surveys, usually conducted on a representative sample of individuals. Some surveys are based on food-frequency, dietary-history questionnaires or household purchases without cover longer periods of times in term of individual dietary consumption (Dorne et al., 2009). Geographical and cultural differences can influence exposure levels of COPs. Because of their diet rich in fats and meat products, the U.S. population may be exposed to high cholesterol diet, and therefore its oxidative products, at a very early age. In addition, individuals and particular subpopulations (i.e. infants, children and elder people) may be exposed or respond differently to these chemicals (Cote et al., 2016). Datasets regarding exposure and effects are needed to evaluate the dose-response (toxicological assessment) and further risk characterization of COPs. For a *priori hazard assessment* a dietary exposure model should be performed. A combination of exposure (total dietary intake) and kinetic modelling (dose-depending activity) could give the initial hints in the estimation of human exposure to COPs. Informing industry members, stakeholders and federal agencies of the dietary intake of COPs and their negative health effects is crucial to take the proper actions in reduce its production during the whole food chain.

### 4.2. COPs as biomarkers of food safety

Several *omics* (i.e. metabolomics, lipidomics, and nutrigenomics, among others) have been already applied to characterize postharvest metabolite heterogeneity of fruits (Pedreschi et al., 2014), food toxins (Giacometti et al., 2013), and nutritional components (Vergères, 2013) for studying biomarkers (Bordoni and Capozzi, 2015), as well as metabolic pathways (Jain et al., 2015). In the food area, foodomics and metabolomics profiling have been mainly focused in food quality, safety and nutrition (Kim et al., 2016); nonetheless the application of *omics* to evaluate and trace effects of processing as fingerprint of the process operations themselves is scarcely used.

The assessment of dietary level of cholesterol and its derivatives combines dietary consumption data with occurrence. Cholesterol information should be found in the nutritional label that the FDA requests for the foods products derivatives. This information may be insufficient for a consistent and systematic monitoring of COPs content in food, to support a risk assessment study. Challenges in cholesterol and COPs quantitation has been reduced by applying *omics* approaches to the field, i.e. *cholesterolomics*, a field that is dedicated to the extraction, isolation and quantification of the *cholesterome* in food, cells, tissues, organs and biofluids (Griffiths et al., 2017). Chromatographic techniques couple with single (GC-MS) or triple quadrupole (LC-MS/MS) have been enhancing COPs detection and quantification (Chiu et al., 2018; Helmschrodt et al., 2013). 7-Keto has been largely detected and quantified in both model and food systems (Rodriguez-Estrada et al., 2014). During thermal-induced oxidation, hydroperoxides in C-7 are mainly generated, thus 7-keto is one of the most representative COPs in food systems ranging from 30% to 70% of the total content of COPs. This has suggested that 7-keto can be the most reliable biomarker of cholesterol oxidation due high temperature processing of foods (Rodriguez-Estrada et al., 2014). However, other processing such as ultra-high temperature (UHT) pasteurization can also generate side-chain COPs (Pikul et al., 2013), including the demonstrated cytotoxic 25-OH. It is still unclear if COPs composition can reflect specific processing technologies, as some early studies have demonstrated. For example, gamma radiation triggers the specific oxidation of cholesterol in the positions C5 and C6 (Maerker and Jones, 1993; Medina-Meza et al., 2012). However, more mechanistic and quantitative studies for a suitable estimation of COPs in food products, and a harmonization of methodologies for their determination is still needed.

## 5. Paradigm shifts and future challenges

### 5.1. Oxidative mechanisms: holistic approach

Many conditions are responsible of trigging/inhibiting the formation of cholesterol oxidative products in food preparations, as reviewed in Tables 3–7. Given the discussed complexity on the oxidative phenomena in the food matrix, any predictive model that would aim to considers all chemical and biochemical parameters, as well as intrinsic and extrinsic factors, is enormously challenging. Analytical strategy can potentially "dissect" the oxidative process considering a few factors at the time. Biomimetics and model systems have been widely used to reproduce several aspects of the cell or tissues outside a living system and are useful to represent a real-world phenomenon. These experimental settings may help to understand the biochemistry associated to the formation of COPs. Models for effects of heating on cholesterol oxidative product formation (Chien et al., 1998) and for high pressure processing's effects on cholesterol oxidative product formation (Medina-Meza et al., 2014a,b) have been already developed. Further model systems that can account for raw material composition and heterogeneity, processing and storage time and conditions, presence/absence of antioxidants: together, this information could also be very beneficial in assessing COPs risk exposure. On the other hand, cell biomimetics and animal studies can effectively provide information regarding COPs physiological absorption. Model systems not only can help to predict the concentrations of cholesterol oxidative products in certain foods, tissues, cell lines and body fluids but also can be used to further understand human exposure to cholesterol oxides (Egeghy et al., 2016). In our opinion, a holistic approach in the cholesterol and lipid oxidation fields should include targeted and high throughput analytical techniques along with higher order statistical modelling. The application of "processomics" (Grauwet et al., 2014) for fingerprinting of food/biological markers and exposure assessment will potentially address the following questions: What is the association between dietary COPs and the major chronic diseases? Are COPs just food biomarkers? Given the power of holistic and as consequently translational approaches in the cholesterolome development, the improvement of analytical resolution and sensitivity, markers fingerprinting, and modelling is highly encouraged.

## 6. Conclusions

COPs are formed both chemically and enzymatically in foods and the human body. More holistic research is required to assess the contribution of dietary and endogenous sources to the total COPs levels found in animals and humans. The need of a systematic review of the toxicology activities of COPs and the estimation of dialy intake of COPs is mandatory, to understand the connection between diet, food manufacturing and epidemiology, which will be a "key component" in this puzzle. Additionally, the creation of a culture of prevention towards cholesterol and lipid oxidation in foods should be encouraged, together with the preservation of intrinsic natural antioxidants, as a strategy that can dampen the formation and accumulation of COPs in foods.

## Declaration of interest

The authors' affiliation is as shown on the cover page. The authors have sole responsibility for the writing and content of the paper.

*Figure 6: (cont'd)*



**Acknowledgments**

This study has been partially funded by the Center for Research on Ingredient Safety (CRIS) through the grant GR100229 and the ENSURE Program Fellowship to Matthew Schweiss at Michigan State University.

**Transparency document**

Transparency document related to this article can be found online at https://doi.org/10.1016/j.fct.2018.05.059.

**References**

About Chronic Diseases, in: N.H. Council (Ed.), 2014, http://www.nationalhealthcouncil.org/sites/default/files/NHC_Files/Pdf_Files/AboutChronicDisease.pdf.
AA.VV., 2015. Chronic diseases: the leading causes of death and disability in the United States. In: Chronic Disease Overview.
Adamczyk, M., Scherrer, E., Kupferberg, A., Malviya, A.N., Mersel, M., 1998. Inhibition of p42/p44 mitogen-activated protein kinase by oxysterols in rat astrocyte primary cultures and C6 glioma cell lines. J. Neurosci. Res. 53, 38–50.
Al-Ismail, K., 2002. Effect of two methods of grilling on the oxidative rancidity and cholesterol oxidation in beef and chicken shawerma. Grasas Aceites 53, 335–339.
Al-Ismail, K.M., Herzallah, S.M., Humied, M.A., 2007. Effect of processing and storage of Jameed on conjugated linoleic acid content and fat and cholesterol oxidation. LWT - Food Sci. Technol. 40, 454–459.
Aldini, G., Yeum, K.-J., Niki, E., Russell, R.M., 2010. Biomarkers for Antioxidant Defense and Oxidative Damage : Principles and Practical Applications, 1st ed. Wiley.
Alkazemi, D., Egeland, G., Vaya, J., Meltzer, S., Kubow, S., 2008. Oxysterol as a marker of atherogenic dyslipidemia in adolescence. J. Clin. Endocrinol. Metab. 93, 4282–4289.
Angulo, A.J., Romera, J.M., Ramirez, M., Gil, A., 1997. Determination of cholesterol oxides in dairy products. Effect of storage conditions. J. Agric. Food Chem. 45, 4318–4323.
Avsaroglu, M.D., Buzrul, S., Alpas, H., Akcelik, M., Bozoglu, F., 2006. Use of the Weibull model for lactococcal bacteriophage inactivation by high hydrostatic pressure. Int. J. Food Microbiol. 108, 78–83.
Aziz, N.H., Mahrous, S.R., Youssef, B.M., 2002. Effect of gamma-ray and microwave treatment on the shelf-life of beef products stored at 5degreeC. Food Contr. 13, 437–444.
Baggio, S.R., Bragagnolo, N., 2006. Cholesterol oxide, cholesterol, total lipid and fatty acid contents in processed meat products during storage. LWT - Food Sci. Technol. 39, 513–520.
Barnaba, C., Rodriguez-Estrada, M.T., Lercker, G., Garcia, H.S., Medina-Meza, I.G., 2016. Cholesterol photo-oxidation: a chemical reaction network for kinetic modeling. Steroids 116, 52–59.
Barriuso, B., Ansorena, D., Poyato, C., I., Astiasarán, 2015. Cholesterol and stigmasterol within a sunflower oil matrix: thermal degradation and oxysterols formation. Steroids 99, 155–160.
Barriuso, B., Mariutti, L.R.B., Ansorena, D., Astiasarán, I., Bragagnolo, N., 2016. Solanum sessiliflorum (mana-cubiu) antioxidant protective effect toward cholesterol oxidation: influence of docosahexaenoic acid. Eur. J. Lipid Sci. Technol. 118, 1125–1131.
Beltran, E., Pla, R., Capellas, M., Yuste, J., Mor-Mur, M., 2004. Lipid oxidation and colour in pressure- and heat-treated minced chicken thighs. J. Sci. Food Agric. 84, 1285–1289.
Berthier, A., Lemaire-Ewing, S., Prunet, C., Montange, T., Vejux, A., Pais de Barros, J.P., Monier, S., Gambert, P., Lizard, G., Neel, D., 2005. 7-Ketocholesterol-induced apoptosis. Involvement of several pro-apoptotic but also anti-apoptotic calcium-dependent transduction pathways. FEBS J. 272, 3093–3104.
Biasi, F., Chiarpotto, E., Sottero, B., Maina, M., Mascia, C., Guina, T., Gamba, P., Gargiulo, S., Testa, G., Leonarduzzi, G., Poli, G., 2013. Evidence of cell damage induced by major components of a diet-compatible mixture of oxysterols in human colon cancer CaCo-2 cell line. Biochimie 95, 632–640.
Biasi, F., Mascia, C., Astegiano, M., Chiarpotto, E., Nano, M., Vizio, B., Leonarduzzi, G., Poli, G., 2009. Pro-oxidant and proapoptotic effects of cholesterol oxidation products on human colonic epithelial cells: a potential mechanism of inflammatory bowel disease progression. Free Radic. Biol. Med. 47, 1731–1741.
Bilska, A., Rudzińska, M., Kowalski, R., Krysztofiak, K., 2009. The effect of soy hydrolysates on changes in cholesterol content and its oxidation products in fine-ground model sausages. Acta Sci. Pol. Technol. Aliment. 8, 15–22.
Bjorkhem, I., Lovgren-Sandblom, A., Leoni, V., Meaney, S., Brodin, L., Salveson, L., Winge, K., Palhagen, S., Svenningsson, P., 2013. Oxysterols and Parkinson's disease: evidence that levels of 24S-hydroxycholesterol in cerebrospinal fluid correlates with the duration of the disease. Neurosci. Lett. 555, 102–105.
Bordoni, A., Capozzi, F., 2015. The foodomics approach for discovering biomarkers of food consumption in nutrition studies. Curr. Opin. Food Sci. 4, 124–128.
Bosco, D.A., Fowler, D.M., Zhang, Q., Nieva, J., Powers, E.T., Wentworth Jr., P., Lerner, R.A., Kelly, J.W., 2006. Elevated levels of oxidized cholesterol metabolites in Lewy body disease brains accelerate alpha-synuclein fibrilization. Nat. Chem. Biol. 2, 249–253.
Boselli, E., Velazco, V., Caboni, M.F., Lercker, G., 2001. Pressurized liquid extraction of lipids for the determination of oxysterols in egg-containing food. J. Chromatogr. A 917, 239–244.
Bretillon, L., Diczfalusy, U., Bjorkhem, I., Maire, M.A., Martine, L., Joffre, C., Acar, N., Bron, A., Creuzot-Garcher, C., 2007. Cholesterol-24S-hydroxylase (CYP46A1) is specifically expressed in neurons of the neural retina. Curr. Eye Res. 32, 361–366.
Britt, C., Gomaa, E.A., Gray, J.I., Booren, A.M., 1998. Influence of cherry tissue on lipid oxidation and heterocyclic aromatic amine formation in ground beef patties. J. Agric. Food Chem. 46, 4891–4897.
Broncano, J.M., Petron, M.J., Parra, V., Timon, M.L., 2009. Effect of different cooking methods on lipid oxidation and formation of free cholesterol oxidation products (COPs) in Latissimus dorsi muscle of Iberian pigs. Meat Sci. 83, 431–437.
Brzeska, M., Szymczyk, K., Szterk, A., 2016. Current knowledge about oxysterols: a review. J. Food Sci. 81.
Calderón-Santiago, M., Peralbo-Molina, Á., Priego-Capote, F., de Castro, L., Dolores, M., 2012. Cholesterol oxidation products in milk: processing formation and determination. Eur. J. Lipid Sci. Technol. 114, 687–694.
Carpenter, K.L., Kirkpatrick, P.J., Weissberg, P.L., Challis, I.R., Dennis, I.F., Freeman, M.A., Mitchinson, M.J., 2003. Oral alpha-tocopherol supplementation inhibits lipid oxidation in established human atherosclerotic lesions. Free Radic. Res. 37, 1235–1244.
Chen, L.J., Lu, Y.F., Chien, J.T., Chen, B.H., 2010. Formation and inhibition of cholesterol oxidation products in tea-leaf eggs during marinating. J. Agric. Food Chem. 58, 10467–10474.
Chen, Y.C., Chien, J.T., Inbaraj, B.S., Chen, B.H., 2012. Formation and inhibition of cholesterol oxidation products during marinating of pig feet. J. Agric. Food Chem. 60, 173–179.
Chien, J.T., Wang, H.C., Chen, B.H., 1998. Kinetic model of the cholesterol oxidation during heating. J. Agric. Food Chem. 46, 2572–2577.
Chiu, C.W., Kao, T.H., Chen, B.H., 2018. Improved analytical method for determination of cholesterol-oxidation products in meat and animal fat by QuEChERS coupled with gas chromatography-mass spectrometry. J. Agric. Food Chem. 66, 3561–3571.
Clariana, M., Garcia-Regueiro, J.A., 2011. Effect of high pressure processing on cholesterol oxidation products in vacuum packaged sliced dry-cured ham. Food Chem. Toxicol. 49, 1468–1471.
Colles, S.M., Maxson, J.M., Carlson, S.G., Chisolm, G.M., 2001. Oxidized LDL-induced injury and apoptosis in atherosclerosis. Potential roles for oxysterols. Trends Cardiovasc. Med. 11, 131–138.
Cote, I., Andersen, M.E., Ankley, G.T., Barone, S., Birnbaum, L.S., Boekelheide, K., Bois, F.Y., Burgoon, L.D., Chiu, W.A., Crawford-Brown, D., Crofton, K.M., DeVito, M., Devlin, R.B., Edwards, S.W., Guyton, K.Z., Hattis, D., Judson, R.S., Knight, D., Krewski, D., Lambert, J., Maull, E.A., Mendrick, D., Paoli, G.M., Patel, C.J., Perkins, E.J., Poje, G., Portier, C.J., Rusyn, I., Schulte, P.A., Simeonov, A., Smith, M.T., Thayer, K.A., Thomas, R.S., Thomas, R., Tice, R.R., Vandenberg, J.J., Villeneuve, D.L., Wesselkamper, S., Whelan, M., Whittaker, C., White, R., Xia, M., Yauk, C., Zeise, L., Zhao, J., DeWoskin, R.S., 2016. The next generation of risk assessment multi-year study-highlights of findings, applications to risk assessment, and future directions. Environ. Health Perspect. 124, 1671–1682.
Cruz, P., Torres, C., Ramirez, M.E., Epunan, M.J., Valladares, L.E., Sierralta, W.D., 2010. Proliferation of human mammary cancer cells exposed to 27-hydroxycholesterol. Exp. Ther. Med. 1, 531–536.
Damjanovic Desic, S., Birlouez-Aragon, I., 2011. The FAST index – a highly sensitive indicator of the heat impact on infant formula model. Food Chem. 124, 1043–1049.
Del Rio, D., Rodriguez-Mateos, A., Spencer, J.P., Tognolini, M., Borges, G., Crozier, A., 2013. Dietary (poly)phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases. Antioxid. Redox Sign. 18, 1818–1892.
Derewiaka, D., Molinska nee Sosinska, E., 2015. Cholesterol transformations during heat treatment. Food Chem. 171, 233–240.
Derewiaka, D., Obiedzinski, M., 2010. Influence of lard heat treatment on changes in the content of cholesterol and formation of cholesterol oxidation products. Pol. J. Food Nutr. Sci. 60 (8).
Derewiaka, D., Obiedziński, M., 2009. Oxysterol content in selected meats and meat products. Acta Scientiarum Polonorum Technologia Alimentaria 8, 5–13.
Derewiaka, D., Obiedziński, M., 2010. Cholesterol oxides content in selected animal products determined by GC-MS. Eur. J. Lipid Sci. Technol. 112, 1130–1137.
Dionisi, F., Golay, P., Aeschlimann, J., Fay, L., 1998. Determination of cholesterol oxidation products in milk powders: methods comparison and validation. J. Agric. Food Chem. 46, 2227–2233.
Dorne, J.L.C.M., Dorne, J.L.C.M., Bordajandi, L.R., Amzal, B., Ferrari, P., Verger, P., 2009. Combining analytical techniques, exposure assessment and biological effects for risk assessment of chemicals in food. Trac. Trends Anal. Chem. 28, 695–707.
Du, M., Ahn, D.U., 2000. Effects of antioxidants and packaging on lipid and cholesterol oxidation and color changes of irradiated egg yolk powder. J. Food Sci. 65, 625–629.
Echarte, M., Conchillo, A., Ansorena, D., Astiasarán, I., 2004. Evaluation of the nutritional aspects and cholesterol oxidation products of pork liver and fish patés. Food Chem. 86, 47–53.
Egeghy, P.P., Sheldon, L.S., Isaacs, K.K., Ozkaynak, H., Goldsmith, M.R., Wambaugh, J.F., Judson, R.S., Buckley, T.J., 2016. Computational exposure science: an emerging discipline to support 21st-century risk assessment. Environ. Health Perspect. 124, 697–702.
Emanuel, H.A., Hassel, C.A., Addis, P.B., Bergmann, S.D., Zavoral, J.H., 1991. Plasma cholesterol oxidation products (oxysterols) in human subjects fed a meal rich in oxysterols. J. Food Sci. 56, 843–847.
FDA, 2014. Total dietary study. In: Research, F.F.S. (Ed.), Food Science Research, . http://www.fda.gov/Food/FoodScienceResearch/TotalDietStudy/ucm184232.htm.
FoodSafety, 2017. Meat and Poultry Roasting Chart. Foodsafety.gov. https://www.foodsafety.gov/keep/charts/meatchart.html.
Fukuchi, J., Hiipakka, R.A., Kokontis, J.M., Hsu, S., Ko, A.L., Fitzgerald, M.L., Liao, S., 2004. Androgenic suppression of ATP-binding cassette transporter A1 expression in

*Figure 6: (cont'd)*



LNCaP human prostate cancer cells. Canc. Res. 64, 7682–7685.
Garcia-Cruset, S., Carpenter, K.L., Guardiola, F., Stein, B.K., Mitchinson, M.J., 2001. Oxysterol profiles of normal human arteries, fatty streaks and advanced lesions. Free Radic. Res. 35, 31–41.
Gargiulo, S., Gamba, P., Testa, G., Leonarduzzi, G., Poli, G., 2016. The role of oxysterols in vascular ageing. J. Physiol. 594, 2095–2113.
Gargiulo, S., Gamba, P., Testa, G., Rossin, D., Biasi, F., Poli, G., Leonarduzzi, G., 2015. Relation between TLR4/NF-kappaB signaling pathway activation by 27-hydroxycholesterol and 4-hydroxynonenal, and atherosclerotic plaque instability. Aging Cell 14, 569–581.
Giacometti, J., Tomljanović, A.B., Josić, D., 2013. Application of proteomics and metabolomics for investigation of food toxins. Food Res. Int. 54, 1042–1051.
Girao, H., Mota, M.C., Ramalho, J., Pereira, P., 1998. Cholesterol oxides accumulate in human cataracts. Exp. Eye Res. 66, 645–652.
Gorassini, A., Verardo, G., Fregolent, S.C., Bortolomeazzi, R., 2017. Rapid determination of cholesterol oxidation products in milk powder based products by reversed phase SPE and HPLC-APCI-MS/MS. Food Chem. 230, 604–610.
Grandgirard, A., Guardiola, F., Dutta, P., Codony, R., Savage, G., 2002. Biological effects of phytosterol oxidation products, future research areas and concluding remarks. Cholesterol and phytosterol oxidation products: analysis, occurrence, and biological effects 375–382.
Grau, A., Codony, R., Grimpa, S., Baucells, M.D., Guardiola, F., 2001. Cholesterol oxidation in frozen dark chicken meat: influence of dietary fat source, and alpha-tocopherol and ascorbic acid supplementation. Meat Sci. 57, 197–208.
Grauwet, T., Vervoort, L., Colle, I., Van Loey, A., Hendrickx, M., 2014. From fingerprinting to kinetics in evaluating food quality changes. Trends Biotechnol. 32, 125–131.
Griffiths, W.J., Abdel-Khalik, J., Yutuc, E., Morgan, A.H., Gilmore, I., Hearn, T., Wang, Y., 2017. Cholesterolomics: an update. Anal. Biochem. 524, 56–67.
Gruenke, L.D., Wrensch, M.R., Petrakis, N.L., Miike, R., Ernster, V.L., Craig, J.C., 1987. Breast fluid cholesterol and cholesterol epoxides: relationship to breast cancer risk factors and other characteristics. Canc. Res. 47, 5483–5487.
Guardiola, F., 1994. Formation of Oxysterols in Egg Powder during spray-drying and Storage. Universitat de Barcelona, Barcelona, Spain.
Guardiola, F., Dutta, P.C., Codony, R., Savage, G.P., 2002. Cholesterol and Phytosterol Oxidation Products: Analysis, Occurrence, and Biological Effects. AOCS Press, Champaign, IL.
Hanlon, P., Brorby, G.P., Krishan, M., 2016. A risk-based strategy for evaluating mitigation options for process-formed compounds in food: workshop proceedings. Int. J. Toxicol. 35, 358–370.
Helmschrodt, C., Becker, S., Schroter, J., Hecht, M., Aust, G., Thiery, J., Ceglarek, U., 2013. Fast LC-MS/MS analysis of free oxysterols derived from reactive oxygen species in human plasma and carotid plaque. Clin. Chim.Acta 425, 3–8.
Hernandez Becerra, J.A., Ochoa Flores, A.A., Valerio-Alfaro, G., Soto-Rodriguez, I., Rodriguez-Estrada, M.T., Garcia, H.S., 2014. Cholesterol oxidation and astaxanthin degradation in shrimp during sun drying and storage. Food Chem. 145, 832–839.
Herzallah, S.M., 2005. Influence of microwaving and conventional heating of milk on cholesterol. Pakistan J. Nutr. 4, 85–88.
Homma, Y., Kondo, Y., Kaneko, M., Kitamura, T., Nyou, W.T., Yanagisawa, M., Yamamoto, Y., Kakizoe, T., 2004. Promotion of carcinogenesis and oxidative stress by dietary cholesterol in rat prostate. Carcinogenesis 25, 1011–1014.
Huang, L.H., Elvington, A., Randolph, G.J., 2015. The role of the lymphatic system in cholesterol transport. Front. Pharmacol. 6, 182.
Hur, S.J., Lee, S.Y., Lee, S.J., 2015. Effect of biopolymer encapsulation on the digestibility of lipid and cholesterol oxidation products in beef during in vitro human digestion. Food Chem. 166, 254–260.
Hur, S.J., Park, G.B., Joo, S.T., 2007a. A comparison of the effects of dietary conjugated linoleic acid contents, cholesterol, lipid oxidation and drip loss in pork loin and chicken breast. J. Muscle Foods 18, 264–275.
Hur, S.J., Park, G.B., Joo, S.T., 2007b. Formation of cholesterol oxidation products (COPs) in animal products. Food Contr. 18, 939–947.
Ikegami, T., Honda, A., Miyazaki, T., Kohjima, M., Nakamuta, M., Matsuzaki, Y., 2014. Increased serum oxysterol concentrations in patients with chronic hepatitis C virus infection. Biochem. Biophys. Res. Commun. 446, 736–740.
Iuliano, L., 2011. Pathways of cholesterol oxidation via non-enzymatic mechanisms. Chem. Phys. Lipids 164, 457–468.
Iuliano, L., Micheletta, F., Natoli, S., Ginanni Corradini, S., Iappelli, M., Elisei, W., Giovannelli, L., Violi, F., Diczfalusy, U., 2003. Measurement of oxysterols and alpha-tocopherol in plasma and tissue samples as indices of oxidant stress status. Anal. Biochem. 312, 217–223.
Jain, N.S., Dürr, U.H.N., Ramamoorthy, A., 2015. Bioanalytical methods for metabolomic profiling: detection of head and neck cancer, including oral cancer. Chin. Chem. Lett. 26, 407–415.
Javitt, N.B., Javitt, J.C., 2009. The retinal oxysterol pathway: a unifying hypothesis for the cause of age-related macular degeneration. Curr. Opin. Ophthalmol. 20, 151–157.
Jo, C., Ahn, D.U., Lee, J.I., 1999. Lipid and cholesterol oxidation, color changes, and volatile production in irradiated raw pork batters with different fat content. J. Food Qual. 22 (6), 641–651.
Jusakul, A., Yongvanit, P., Loilome, W., Namwat, N., Kuver, R., 2011. Mechanisms of oxysterol-induced carcinogenesis. Lipids Health Dis. 10, 44.
Kang, K.A., Chae, S., Lee, K.H., Park, M.T., Lee, S.J., Lee, Y.S., Hyun, J.W., 2005. Cytotoxic effect of 7beta-hydroxycholesterol on human NCI-H460 lung cancer cells. Biol. Pharm. Bull. 28, 1377–1380.
Khan, M.I., Min, J.S., Lee, S.O., Yim, D.G., Seol, K.H., Lee, M., Jo, C., 2015. Cooking, storage, and reheating effect on the formation of cholesterol oxidation products in processed meat products. Lipids Health Dis. 14, 89.
Kim, S., Kim, J., Yun, E.J., Kim, K.H., 2016. Food metabolomics: from farm to human. Curr. Opin. Biotechnol. 37, 16–23.
Kreilaus, F., Spiro, A.S., McLean, C.A., Garner, B., Jenner, A.M., 2016. Evidence for altered cholesterol metabolism in Huntington's disease post mortem brain tissue. Neuropathol. Appl. Neurobiol. 42, 535–546.
Kulig, W., Cwiklik, L., Jurkiewicz, P., Rog, T., Vattulainen, I., 2016. Cholesterol oxidation products and their biological importance. Chem. Phys. Lipids 199, 144–160.
Kumar, N., Singhal, O.P., 1992. Effect of processing conditions on the oxidation of cholesterol in ghee. J. Sci. Food Agric. 58, 267–273.
Kuver, R., 2012. Mechanisms of oxysterol-induced disease: insights from the biliary system. Clin. Lipidol. 7, 537–548.
Lappano, R., Recchia, A.G., De Francesco, E.M., Angelone, T., Cerra, M.C., Picard, D., Maggiolini, M., 2011. The cholesterol metabolite 25-hydroxycholesterol activates estrogen receptor alpha-mediated signaling in cancer cells and in cardiomyocytes. PLoS One 6, e16631.
Lee, C.Y., Seet, R.C., Huang, S.H., Long, L.H., Halliwell, B., 2009. Different patterns of oxidized lipid products in plasma and urine of dengue fever, stroke, and Parkinson's disease patients: cautions in the use of biomarkers of oxidative stress. Antioxid. Redox Sign. 11, 407–420.
Lee, T.S., Chau, L.Y., 2001. Fas/Fas ligand-mediated death pathway is involved in oxLDL-induced apoptosis in vascular smooth muscle cells. Am. J. Physiol. Cell Physiol. 280, C709–C718.
Lemaire-Ewing, S., Prunet, C., Montange, T., Vejux, A., Berthier, A., Bessede, G., Corcos, L., Gambert, P., Neel, D., Lizard, G., 2005. Comparison of the cytotoxic, pro-oxidant and pro-inflammatory characteristics of different oxysterols. Cell Biol. Toxicol. 21, 97–114.
Lemaire, S., Lizard, G., Monier, S., Miguet, C., Gueldry, S., Volot, F., Gambert, P., Néel, D., 1998. Different patterns of IL-1β secretion, adhesion molecule expression and apoptosis induction in human endothelial cells treated with 7α-, 7β-hydroxycholesterol, or 7-ketocholesterol. FEBS Lett. 440, 434–439.
Leonarduzzi, G., Gargiulo, S., Gamba, P., Perrelli, M.G., Castellano, I., Sapino, A., Sottero, B., Poli, G., 2010. Molecular signaling operated by a diet-compatible mixture of oxysterols in up-regulating CD36 receptor in CD68 positive cells. Mol. Nutr. Food Res. 54 (Suppl. 1), S31–S41.
Leonarduzzi, G., Poli, G., Sottero, B., Biasi, F., 2007. Activation of the mitochondrial pathway of apoptosis by oxysterols. Front. Biosci. 12, 791–799.
Leonarduzzi, G., Sottero, B., Verde, V., Poli, G., Preedy, V., Watson, R., 2005. Oxidized products of cholesterol: toxic effect. Reviews in food and nutrition toxicity 3.
Leoni, V., Caccia, C., 2011. Oxysterols as biomarkers in neurodegenerative diseases. Chem. Phys. Lipids 164, 515–524.
Leoni, V., Long, J.D., Mills, J.A., Di Donato, S., Paulsen, J.S., group, P.-H.s, 2013. Plasma 24S-hydroxycholesterol correlation with markers of Huntington disease progression. Neurobiol. Dis. 55, 37–43.
Leoni, V., Mariotti, C., Nanetti, L., Salvatore, E., Squitieri, F., Bentivoglio, A.R., Bandettini di Poggio, M., Piacentini, S., Monza, D., Valenza, M., Cattaneo, E., Di Donato, S., 2011. Whole body cholesterol metabolism is impaired in Huntington's disease. Neurosci. Lett. 494, 245–249.
Leoni, V., Mariotti, C., Tabrizi, S.J., Valenza, M., Wild, E.J., Henley, S.M., Hobbs, N.Z., Mandelli, M.L., Grisoli, M., Bjorkhem, I., Cattaneo, E., Di Donato, S., 2008. Plasma 24S-hydroxycholesterol and caudate MRI in pre-manifest and early Huntington's disease. Brain 131, 2851–2859.
Li, N., Ohshima, T., Shozen, K.-i, Ushio, H., Koizumi, C., 1994. Effects of the degree of unsaturation of coexisting triacylglycerols on cholesterol oxidation. J. Am. Oil Chem. Soc. 71, 623–627.
Li, S.X., Cherian, G., Ahn, D.U., Hardin, R.T., Sim, J.S., 1996. Storage, heating, and tocopherols affect cholesterol oxide formation in food oils. J. Agric. Food Chem. 44, 3830–3834.
Linseisen, J., Wolfram, G., Miller, A.B., 2002. Plasma 7beta-hydroxycholesterol as a possible predictor of lung cancer risk. Cancer Epidemiol. Biomark. Prev. 11, 1630–1637.
Liu, H., Yuan, L., Xu, S., Wang, K., Zhang, T., 2005. Cholestane-3beta,5alpha,6beta-triol inhibits osteoblastic differentiation and promotes apoptosis of rat bone marrow stromal cells. J. Cell. Biochem. 96, 198–208.
Liu, Y., Hulten, L.M., Wiklund, O., 1997. Macrophages isolated from human atherosclerotic plaques produce IL-8, and oxysterols may have a regulatory function for IL-8 production. Arterioscler. Thromb. Vasc. Biol. 17, 317–323.
Liu, Z., Rochfort, S., Cocks, B.G., 2016. Optimization of a single phase method for lipid extraction from milk. J. Chromatogr. A 1458, 145–149.
Lizard, G., Miguet, C., Besséde, G., Monier, S., Gueldry, S., Neel, D., Gambert, P., 2000. Impairment with various antioxidants of the loss of mitochondrial transmembrane potential and of the cytosolic release of cytochrome c occurring during 7-ketocholesterol-induced apoptosis. Free Radic. Biol. Med. 28, 743–753.
Lopez, J., Tait, S.W., 2015. Mitochondrial apoptosis: killing cancer using the enemy within. Br. J. Canc. 112, 957–962.
Lordan, S., Mackrill, J.J., O'Brien, N.M., 2009. Oxysterols and mechanisms of apoptotic signaling: implications in the pathology of degenerative diseases. J. Nutr. Biochem. 20, 321–336.
Lutjohann, D., Papassotiropoulos, A., Bjorkhem, I., Locatelli, S., Bagli, M., Oehring, R.D., Schlegel, U., Jessen, F., Rao, M.L., von Bergmann, K., Heun, R., 2000. Plasma 24S-hydroxycholesterol (cerebrosterol) is increased in Alzheimer and vascular demented patients. J. Lipid Res. 41, 195–198.
Maerker, G., Jones, K.C., 1993. A-ring oxidation products from γ-irradiation of cholesterol in liposomes. J. Am. Oil Chem. Soc. 70, 255–259.
Mariutti, L.R., Bragagnolo, N., 2017. Influence of salt on lipid oxidation in meat and seafood products: a review. Food Res. Int. 94, 90–100.
Martin, C.R., Ling, P.R., Blackburn, G.L., 2016. Review of infant feeding: key features of

*Figure 6: (cont'd)*



breast milk and infant formula. Nutrients 8.
Marwarha, G., Ghribi, O., 2015. Does the oxysterol 27-hydroxycholesterol underlie Alzheimer's disease-Parkinson's disease overlap? Exp. Gerontol. 68, 13–18.
Mast, N., Annalora, A.J., Lodowski, D.T., Palczewski, K., Stout, C.D., Pikuleva, I.A., 2011. Structural basis for three-step sequential catalysis by the cholesterol side chain cleavage enzyme CYP11A1. J. Biol. Chem. 286, 5607–5613.
Mazalli, M.R., Bragagnolo, N., 2007. Effect of storage on cholesterol oxide formation and fatty acid alterations in egg powder. J. Agric. Food Chem. 55, 2743–2748.
Mazalli, M.R., Bragagnolo, N., 2009. Increase of cholesterol oxidation and decrease of PUFA as a result of thermal processing and storage in eggs enriched with n-3 fatty acids. J. Agric. Food Chem. 57, 5028–5034.
McCluskey, S., Devery, R., 1993. Validation of chromatographic analysis of cholesterol oxides in dried foods. Trends Food Sci. Technol. 4, 175–178.
Medina-Meza, I.G., Barnaba, C., 2013. Kinetics of cholesterol oxidation in model systems and foods: current status. Food Eng. Rev. 5, 171–184.
Medina-Meza, I.G., Barnaba, C., Barbosa-Cánovas, G.V., 2014a. Effects of high pressure processing on lipid oxidation: a review. Innov. Food Sci. Emerg. Technol. 22, 1–10.
Medina-Meza, I.G., Rodríguez-Estrada, M.T., García, H.S., Lercker, G., 2012. Oxidative pattern from fluorescent light exposition of crystalline cholesterol. Food Biophys. 7, 209–219.
Medina-Meza, I.G., Rodriguez-Estrada, M.T., Lercker, G., Soto-Rodriguez, I., Garcia, H.S., 2011. Unusual oxidative pattern in thermo-oxidation of dry films of cholesterol. Rev. Mex. Ing. Quim. 10, 47–57.
Medina-Meza, I.G., Rodriguez-Estrada, M.T., Lercker, G., Barnaba, C., García, H.S., 2014b. Kinetics of 25-hydroperoxycholesterol formation during photo-oxidation of crystalline cholesterol. J. Sci. Food Agric. 94, 1543–1551.
Meister, N., Aebischer, J., Vikas, M., Eyer, K., De Pasquale, D., 2000. Spray-drying Process. (Google Patents).
Menendez-Carreno, M., Varo, N., Mugueta, C., Restituto, P., Ansorena, D., Astiasaran, I., 2011. Correlation between serum content of the main COPs (cholesterol oxidation products) from autoxidation and cardiovascular risk factors. Nutr. Hosp. 26, 144–151.
Micheletta, F., Natoli, S., Misuraca, M., Sbarigia, E., Diczfalusy, U., Iuliano, L., 2004. Vitamin E supplementation in patients with carotid atherosclerosis: reversal of altered oxidative stress status in plasma but not in plaque. Arterioscler. Thromb. Vasc. Biol. 24, 136–140.
Min, J.S., Khan, M.I., Lee, S.O., Yim, D.G., Seol, K.H., Lee, M., Jo, C., 2016. Impact of cooking, storage, and reheating conditions on the formation of cholesterol oxidation products in pork loin. Korean J. Food Sci. Anim. Resour. 36, 23–28.
Min, J.S., Lee, S.O., Khan, M.I., Yim, D.G., Seol, K.H., Lee, M., Jo, C., 2015. Monitoring the formation of cholesterol oxidation products in model systems using response surface methodology. Lipids Health Dis. 14, 77.
Miyoshi, N., Iuliano, L., Tomono, S., Ohshima, H., 2014. Implications of cholesterol autoxidation products in the pathogenesis of inflammatory diseases. Biochem. Biophys. Res. Commun. 446, 702–708.
Moog, C., Deloulme, J., Baudier, J., Revel, M., Bischoff, P., Hietter, H., Luu, B., 1991. Membrane-related oxysterol function: preliminary results on the modification of protein kinase C activity and substrate phosphorylation by 7ß, 25-dihydroxycholesterol. Biochimie 73, 1321–1326.
Mukhopadhyay, S., Fellows, K., Browne, R.W., Khare, P., Krishnan Radhakrishnan, S., Hagemeier, J., Weinstock-Guttman, B., Zivadinov, R., Ramanathan, M., 2017. Interdependence of oxysterols with cholesterol profiles in multiple sclerosis. Mult. Scler. 23, 792–801.
Murakami, H., Tamasawa, N., Matsui, J., Yamato, K., JingZhi, G., Suda, T., 2001. Plasma levels of soluble vascular adhesion molecule-1 and cholesterol oxidation product in type 2 diabetic patients with nephropathy. J. Atherosclerosis Thromb. 8, 21–24.
Nelson, E.R., Chang, C.Y., McDonnell, D.P., 2014. Cholesterol and breast cancer pathophysiology. Trends Endocrinol. Metabol. 25, 649–655.
Nielsen, J.H., Olsen, C.E., Duedahl, C., Skibsted, L.H., 1995. Isolation and quantification of cholesterol oxides in dairy products by selected ion monitoring mass spectrometry. J. Dairy Res. 62, 101–113.
Nielsen, J.H., Olsen, C.E., Jensen, C., Skibsted, L.H., 1996a. Cholesterol oxidation in butter and dairy spread during storage. J. Dairy Res. 63, 159–167.
Nielsen, J.H., Olsen, C.E., Lyndon, J., Sørensen, J., Skibsted, L.H., 1996b. Cholesterol oxidation in feta cheese produced from high-temperature bleached and from non-bleached butteroil from bovine milk. J. Dairy Res. 63, 615–621.
NIH, 2017. High Blood Pressure. U.S. National Library of Medicine.
Nourooz-Zadeh, J., Appelqvist, L.-Å., 1988. Cholesterol oxides in Swedish foods and food ingredients: butter and cheese. J. Am. Oil Chem. Soc. 65, 1635–1641.
Novelli, E., Zanardi, E., Ghiretti, G.P., Campanini, G., Dazzi, G., Madarena, G., Chizzolini, R., 1998. Lipid and cholesterol oxidation in frozen stored pork, salame Milano and mortadella. Meat Sci. 48, 29–40.
Ogino, Y., Osada, K., Nakamura, S., Ohta, Y., Kanda, T., Sugano, M., 2007. Absorption of dietary cholesterol oxidation products and their downstream metabolic effects are reduced by dietary apple polyphenols. Lipids 42, 151–161.
Ohshima, T., Li, N., Koizumi, C., 1993. Oxidative decomposition of cholesterol in fish products. J. Am. Oil Chem. Soc. 70, 595–600.
Olkkonen, V.M., Béaslas, O., Nissilä, E., 2012. Oxysterols and their cellular effectors. Biomolecules 2, 76.
Olkkonen, V.M., Gylling, H., Ikonen, E., 2017. Plant sterols, cholesterol precursors and oxysterols: minute concentrations-Major physiological effects. J. Steroid Biochem. Mol. Biol. 169, 4–9.
Osada, K., Hoshina, S., Nakamura, S., Sugano, M., 2000. Cholesterol oxidation in meat products and its regulation by supplementation of sodium nitrite and apple polyphenol before processing. J. Agric. Food Chem. 48, 3823–3829.
Osada, K., Kodama, T., Cui, L., Yamada, K., Sugano, M., 1993. Levels and formation of oxidized cholesterols in processed marine foods. J. Agric. Food Chem. 41, 1893–1898.
Osada, K., Sasaki, E., Sugano, M., 2014. Inhibition of cholesterol absorption by oxidized cholesterol in rats. Biosci. Biotechnol. Biochem. 58, 782–783.
Overholt, M.F., Mancini, S., Galloway, H.O., Preziuso, G., Dilger, A.C., Boler, D.D., 2016. Effects of salt purity on lipid oxidation, sensory characteristics, and textural properties of fresh, ground pork patties. LWT - Food Sci. Technol. 65, 890–896.
Park, S.W., Addis, P., 1985. HPLC determination of C-7 oxidized cholesterol derivatives in foods. J. Food Sci. 50, 1437–1441.
Pedreschi, R., Muñoz, P., Robledo, P., Becerra, C., Defilippi, B.G., van Eekelen, H., Mumm, R., Westra, E., de Vos, R.C.H., 2014. Metabolomics analysis of postharvest ripening heterogeneity of 'Hass' avocadoes. Postharvest Biol. Technol. 92, 172–179.
Petrón, M.J., García-Regueiro, J.A., Martín, L., Muriel, E., Antequera, T., 2003. Identification and quantification of cholesterol and cholesterol oxidation products in different types of Iberian hams. J. Agric. Food Chem. 51, 5786–5791.
Pickett-Bernard, D., 2006. Infant formula: evaluating the safety of new ingredients. J. Hum. Lactation 22, 231–232.
Picouet, P.A., Fernandez, A., Serra, X., Sunol, J.J., Arnau, J., 2007. Microwave heating of cooked pork patties as a function of fat content. J. Food Sci. 72, E57–E63.
Pie, J.E., Spahis, K., Seillan, C., 1991. Cholesterol oxidation in meat products during cooking and frozen storage. J. Agric. Food Chem. 39, 250–254.
Pikul, J., Rudzińska, M., Teichert, J., Lasik, A., Danków, R., Przybylski, R., 2013. Cholesterol oxidation during storage of UHT-treated bovine and caprine milk. Int. Dairy J. 30, 29–32.
Prunet, C., Lemaire-Ewing, S., Ménétrier, F., Néel, D., Lizard, G., 2005. Activation of caspase-3-dependent and-independent pathways during 7-ketocholesterol-and 7β-hydroxycholesterol-induced cell death: a morphological and biochemical study. J. Biochem. Mol. Toxicol. 19, 311–326.
Przygonski, K., Jelen, H., Wasowicz, E., 2000. Determination of cholesterol oxidation products in milk powder and infant formulas by gas chromatography and mass spectrometry. Nahrung 44, 122–125.
Raza, S., Meyer, M., Schommer, J., Hammer, K.D., Guo, B., Ghribi, O., 2016. 27-Hydroxycholesterol stimulates cell proliferation and resistance to docetaxel-induced apoptosis in prostate epithelial cells. Med. Oncol. 33, 12.
Rey, A.I., Lopez-Bote, C.J., Buckley, J.D., 2004. Effect of feed on cholesterol concentration and oxidation products development of longissimus dorsi muscle from Iberian pigs. Ir. J. Agric. Food Res. 43, 69–83.
Riendeau, V., Garenc, C., 2009. Effect of 27-hydroxycholesterol on survival and death of human macrophages and vascular smooth muscle cells. Free Radic. Res. 43, 1019–1028.
Rodriguez-Estrada, M.T., Garcia-Llatas, G., Lagarda, M.J., 2014. 7-Ketocholesterol as marker of cholesterol oxidation in model and food systems: when and how. Biochem. Biophys. Res. Commun. 446, 792–797.
Roldan, M., Antequera, T., Armenteros, M., Ruiz, J., 2014. Effect of different temperature-time combinations on lipid and protein oxidation of sous-vide cooked lamb loins. Food Chem. 149, 129–136.
Romeu-Nadal, M., Chávez-Servín, J.L., Castellote, A.I., Rivero, M., López-Sabater, M.C., 2007. Oxidation stability of the lipid fraction in milk powder formulas. Food Chem. 100, 756–763.
Ros, E., 2000. Intestinal absorption of triglyceride and cholesterol. Dietary and pharmacological inhibition to reduce cardiovascular risk. Atherosclerosis 151, 357–379.
Rose-Sallin, C., Huggett, A.C., Bosset, J.O., Tabacchi, R., Fay, L.B., 1995. Quantification of cholesterol oxidation products in milk powders using [2H7]Cholesterol to monitor cholesterol autoxidation artifacts. J. Agric. Food Chem. 43, 935–941.
Roussi, S., Winter, A., Gosse, F., Werner, D., Zhang, X., Marchioni, E., Geoffroy, P., Miesch, M., Raul, F., 2005. Different apoptotic mechanisms are involved in the antiproliferative effects of 7beta-hydroxysitosterol and 7beta-hydroxycholesterol in human colon cancer cells. Cell Death Differ. 12, 128–135.
Rozner, S., Garti, N., 2006. The activity and absorption relationship of cholesterol and phytosterols. Colloid. Surface. Physicochem. Eng. Aspect. 282, 435–456.
Rudzińska, M., Flaczyk, E., Amarowicz, R., Wąsowicz, E., Korczak, J., 2006. Antioxidative effect of crackling hydrolysates during frozen storage of cooked pork meatballs. Eur. Food Res. Technol. 224, 293–299.
Sander, B., Smith, D., Addis, P., Park, S., 1989a. Effects of prolonged and adverse storage conditions on levels of cholesterol oxidation products in dairy products. J. Food Sci. 54, 874–879.
Sander, B.D., Addis, P.B., Park, S.W., Smith, D.E., 1989b. Quantification of cholesterol oxidation products in a variety of foods. J. Food Protect. 52, 109–114.
Sarantinos, J., 1993. Identification and Quantification of Cholesterol Oxides in Foods. Deakin University.
Savage, G.P., Dutta, P.C., Rodriguez-Estrada, M.T., 2002. Cholesterol oxides: their occurrence and methods to prevent their generation in foods. Asia Pac. J. Clin. Nutr. 11, 72–78.
Schmarr, H.-G., Gross, H.B., Shibamoto, T., 1996. Analysis of polar cholesterol oxidation products: evaluation of a new method involving transesterification, solid phase extraction, and gas chromatography. J. Agric. Food Chem. 44, 512–517.
Scopesi, F., Zunin, P., Mazzella, M., Testa, M., Boggia, R., Evangelisti, F., Serra, G., 2002. 7-ketocholesterol in human and adapted milk formulas. Clin. Nutr. 21, 379–384.
Seet, R.C., Lee, C.Y., Lim, E.C., Quek, A.M., Yeo, L.L., Huang, S.H., Halliwell, B., 2009. Oxidative damage in dengue fever. Free Radic. Biol. Med. 47, 375–380.
Seimon, T., Tabas, I., 2009. Mechanisms and consequences of macrophage apoptosis in atherosclerosis. J. Lipid Res. 50 (Suppl. l), S382–S387.
Serra, A., Conte, G., Cappucci, A., Casarosa, L., Mele, M., 2016. Cholesterol and fatty acids oxidation in meat from three muscles of massese suckling lambs slaughtered at different weights. Ital. J. Anim. Sci. 13, 3275.
Sevanian, A., Peterson, A.R., 1984. Cholesterol epoxide is a direct-acting mutagen. Proc.

*Figure 6:* *(cont'd)*



Natl. Acad. Sci. U. S. A. 81, 4198–4202.
Sevanian, A., Peterson, A.R., 1986. The cytotoxic and mutagenic properties of cholesterol oxidation products. Food Chem. Toxicol. 24, 1103–1110.
Shim, C.Y., Park, S., Choi, E.Y., Kang, S.M., Cha, B.S., Ha, J.W., Rim, S.J., Lee, H.C., Chung, N., 2008. Is albuminuria an indicator of myocardial dysfunction in diabetic patients without overt heart disease? A study with Doppler strain and strain rate imaging. Metabolism 57, 448–452.
Shozen, K.-i., Ohshima, T., Ushio, H., Koizumi, C., 1995. formation of cholesterol oxides in marine fish products induced by grilling. Fish. Sci. 61, 817–821.
Sieber, R., 2005. Oxidised cholesterol in milk and dairy products. Int. Dairy J. 15, 191–206.
Smith, L.L., 1996. Review of progress in sterol oxidations: 1987-1995. Lipids 31, 453–487.
Sun, X.D., Holley, R.A., 2010. High hydrostatic pressure effects on the texture of meat and meat products. J. Food Sci. 75, R17–R23.
Tai, C.Y., Chen, Y.C., Chen, B.H., 1999. Analysis, formation and inhibition of cholesterol oxidation products in foods: an overview (Part I). J. Food Drug Anal. 7, 243–257.
Tarvainen, M., Nuora, A., Quirin, K.-W., Kallio, H., Yang, B., 2015. Effects of CO 2 plant extracts on triacylglycerol oxidation in Atlantic salmon during cooking and storage. Food Chem. 173, 1011–1021.
Trevisi, L., Bertoldo, A., Agnoletto, L., Poggiani, C., Cusinato, F., Luciani, S., 2010. Antiapoptotic and proliferative effects of low concentrations of 7β-hydroxycholesterol in human endothelial cells via ERK activation. J. Vasc. Res. 47, 241–251.
Tsai, L.S., Hudson, C.A., 2006. Cholesterol oxides in commercial dry egg products: quantitation. J. Food Sci. 50, 229–231.
Ubhayasekera, S.J., Kochhar, S.P., Dutta, P.C., 2006. Lipids and lipid oxidation with emphasis on cholesterol oxides in some Indian sweets available in London. Int. J. Food Sci. Nutr. 57, 451–458.
Upston, J.M., Niu, X., Brown, A.J., Mashima, R., Wang, H., Senthilmohan, R., Kettle, A.J., Dean, R.T., Stocker, R., 2002. Disease stage-dependent accumulation of lipid and protein oxidation products in human atherosclerosis. Am. J. Pathol. 160, 701–710.
Vaughn, D.W., Green, S., Kalayanarooj, S., Innis, B.L., Nimmannitya, S., Suntayakorn, S., Rothman, A.L., Ennis, F.A., Nisalak, A., 1997. Dengue in the early febrile phase: viremia and antibody responses. J. Infect. Dis. 176, 322–330.
Vaya, J., Aviram, M., Mahmood, S., Hayek, T., Grenadir, E., Hoffman, A., Milo, S., 2001. Selective distribution of oxysterols in atherosclerotic lesions and human plasma lipoproteins. Free Radic. Res. 34, 485–497.
Vejux, A., Lizard, G., 2009. Cytotoxic effects of oxysterols associated with human diseases: induction of cell death (apoptosis and/or oncosis), oxidative and inflammatory activities, and phospholipidosis. Mol. Aspect. Med. 30, 153–170.
Verardo, V., Riciputi, Y., Messia, M.C., Marconi, E., Caboni, M.F., 2016. Influence of drying temperatures on the quality of pasta formulated with different egg products. Eur. Food Res. Technol. 243, 817–825.
Vergères, G., 2013. Nutrigenomics – linking food to human metabolism. Trends Food Sci. Technol. 31, 6–12.
Vine, D.F., Croft, K.D., Beilin, L.J., Mamo, J.C.L., 1997. Absorption of dietary cholesterol oxidation products and incorporation into rat lymph chylomicrons. Lipids 32, 887–893.
Vine, D.F., Mamo, J.C., Beilin, L.J., Mori, T., Croft, K.D., 1998. Dietary oxysterols are incorporated in plasma triglyceriderich lipoproteins, increase their susceptibility to oxidation and increase aortic cholesterol concentration of rabbits. J. Lipid Res. 39, 1995–2004.
Virginio, V.W., Nunes, V.S., Moura, F.A., Menezes, F.H., Andreollo, N.A., Rogerio, F., Scherrer, D.Z., Quintao, E.C., Nakandakare, E., Petrucci, O., Nadruz-Junior, W., de Faria, E.C., Sposito, A.C., 2015. Arterial tissue and plasma concentration of enzymatic-driven oxysterols are associated with severe peripheral atherosclerotic disease and systemic inflammatory activity. Free Radic. Res. 49, 199–203.
Vore, V., 1988. TBA values and 7-ketocholesterol in refrigerated raw and cooked ground beef. J. Food Sci. 53, 1058–1061.
Vurusaner, B., Gamba, P., Testa, G., Gargiulo, S., Leonarduzzi, G., Poli, G., Basaga, H., 2016a. Molecular mechanism of oxysterol induced survival response: the role of autophagy and ROS. Free Radic. Biol. Med. 100, S46.
Vurusaner, B., Leonarduzzi, G., Gamba, P., Poli, G., Basaga, H., 2016b. Oxysterols and mechanisms of survival signaling. Mol. Aspect. Med. 49, 8–22.
Wolf, B.B., Green, D.R., 1999. Suicidal tendencies: apoptotic cell death by caspase family proteinases. J. Biol. Chem. 274, 20049–20052.
Wu, Q., Ishikawa, T., Sirianni, R., Tang, H., McDonald, J.G., Yuhanna, I.S., Thompson, B., Girard, L., Mineo, C., Brekken, R.A., Umetani, M., Euhus, D.M., Xie, Y., Shaul, P.W., 2013. 27-Hydroxycholesterol promotes cell-autonomous, ER-positive breast cancer growth. Cell Rep. 5, 637–645.
Xu, L., Korade, Z., Rosado Jr., D.A., Liu, W., Lamberson, C.R., Porter, N.A., 2011. An oxysterol biomarker for 7-dehydrocholesterol oxidation in cell/mouse models for Smith-Lemli-Opitz syndrome. J. Lipid Res. 52, 1222–1233.
Xu, L., Mirnics, K., Bowman, A.B., Liu, W., Da, J., Porter, N.A., Korade, Z., 2012. DHCEO accumulation is a critical mediator of pathophysiology in a Smith-Lemli-Opitz syndrome model. Neurobiol. Dis. 45, 923–929.
Yarmand, M.S., Homayouni, A., 2009. Effect of microwave cooking on the microstructure and quality of meat in goat and lamb. Food Chem. 112, 782–785.
Yoshida, T., Matsuzaki, Y., Haigh, W.G., Fukushima, S., Ikezawa, K., Tanaka, N., Lee, S.P., 2003. Origin of oxysterols in hepatic bile of patients with biliary infection. Am. J. Gastroenterol. 98, 2275–2280.
Zardetto, S., Barbanti, D., Rosa, M.D., 2014. Formation of cholesterol oxidation products (COPs) and loss of cholesterol in fresh egg pasta as a function of thermal treatment processing. Food Res. Int. 62, 177–182.
Zarrouk, A., Vejux, A., Mackrill, J., O'Callaghan, Y., Hammami, M., O'Brien, N., Lizard, G., 2014. Involvement of oxysterols in age-related diseases and ageing processes. Ageing Res. Rev. 18, 148–162.
Zhang, Y., Ye, X., Xu, Z., Duan, J., Wei, C., Xu, G., Chen, S., 2017. Inhibitory effect of proanthocyanidins from Chinese bayberry ( Myrica rubra Sieb. et Zucc. ) leaves on the lipid oxidation in an emulsion system. LWT-Food Sci. Technol. 80, 517–522.
Zubillaga, M., Maerker, G., 1991. Quantification of three cholesterol oxidation products in raw meat and chicken. J. Food Sci. 56, 1194–1196.
Zunin, P., Boggia, R., Evangelisti, F., 2001. Identification and quantification of cholesterol oxidation products in canned tuna. J. Am. Oil Chem. Soc. 78, 1037–1040.
Zunin, P., Salvadeo, P., Boggia, R., Evangelisti, F., 2006. Sterol oxidation in meat- and fish-based homogenized baby foods containing vegetable oils. J. AOAC Int. 89, 441–446.

# APPENDIX B

## PHYTOSTEROLS AND THEIR OXIDATIVE PRODUCTS IN INFANT FORMULA (JOURNAL PAPER)

*Figure 7: Phytosterols and their Oxidative Products in Infant Formula (Journal Paper)*

Received: 19 March 2019 | Revised: 16 May 2019 | Accepted: 20 May 2019
DOI: 10.1111/jfpe.13151

SPECIAL ISSUE REVIEW

Journal of Food Process Engineering WILEY

# Phytosterols and their oxidative products in infant formula

Alice Kilvington[1] | Lisaura Maldonado-Pereira[1] | Cristobal Torres-Palacios[2] | Ilce Medina-Meza[1,2]

[1]Department of Chemical Engineering and Materials Science, Michigan State University, East Lansing, Michigan
[2]Department of Biosystems and Agricultural Engineering, Michigan State University, East Lansing, Michigan

Correspondence
Ilce Medina-Meza, Department of Biosystems and Agricultural Engineering, 469 Wilson Rd.| Room 302C, East Lansing, MI.
Email: ilce@msu.edu

Funding information
Consejo Nacional de Ciencia y Tecnología, Grant/Award Number: 711082; SHORA Spectrum Health, Grant/Award Number: RC108332

**Abstract**

Infant formulations are enriched with vegetable oils that confer not only calories, but also peculiar chemical attributes. Vegetable oils are particularly rich in phytosterols, a class of triterpene molecules analogous to cholesterol, but with different and largely unknown biological effects. The preparation and sterilization of infant formula provide opportune physicochemical conditions for oxidative reactions to occur. Oxidation of phytosterols during the preparation of infant formula can led to oxidized derivatives, known as phytosterol oxidation products [POPs], which harmful effects can be exacerbated given the wide variety of infant formulas characterized by their exclusivity of milk surrogates, required to fulfill specific needs in the nutritional development of a baby. In this review, the state-of-the-art regarding phytosterols and their presence in infant formulation is revised, stressing the need of further investigation in the field of food processing. Reconsidering infant formula manufacturing in the context of phytosterols oxidation will lead to several opportunities for food engineers and technologists in the food safety.

**Practical applications**

Phytosterols are plant-based bioactive lipids with health benefits. Therefore, they have been implemented as a supplement in infant formula through the addition of vegetable oils. Processing, packaging, and storage contribute to the oxidative process of these compounds. A surveillance of the entire food chain is needed to reduce the oxidative load in the final product.

## 1 | PHYTOSTEROLS—VEGETABLE STEROLS

Phytosterols also known as plant sterols (PS), are natural compounds which are members of the triterpene family. The triterpene group includes more than 4,000 compounds and over 100 of those are phytosterols. In food matrices, PS have been chemically characterized and quantified for over three decades (Moreau, Whitaker, & Hicks, 2002). PS are 28- or 29-carbon alcohols with a steroid nucleus, a 3β-hydroxyl group, and a double bond in the C5–C6 position. Compared to cholesterol, PS contain an extra methyl group, ethyl group, or double bond with a side chain of 9–10 carbon atoms in length, instead of a C-8 cholesterol side chain. In plant cells, PS are primarily encountered in the plasma membrane, specifically in the outer membrane of mitochondria and the endoplasmic reticulum. They play an important role as a structural molecule, providing rigidity to the cell membrane by promoting an increase in the sterol/phospholipid ratio that is associated with membrane stiffness (Alemany, Barbera, Alegría, & Laparra, 2014; Comunian & Favaro-Trindade, 2016; Moreau et al., 2002). PS are components of all foods of vegetable origin, and are known for their beneficial property in health of lowering serum total cholesterol concentration, as well as the low-density lipoproteins (LDLs) concentration (Lagarda, García-Llatas, & Farré, 2006). Therefore, for over the

Alice Kilvington and Lisaura Maldonado-Pereira authors equally contributed to the manuscript.

  

*Figure 7* *(cont'd)*



past 15 years, PS have been incorporated into several functional foods as a supplement (García-Llatas & Rodríguez-Estrada, 2011). The structure of the most common phytosterols is shown in Figure 1. The most common phytosterols found in foods are β-sitosterol, campesterol, stigmasterol, and Δ5-avenasterol. β-sitosterol is the most abundant and has a proportion of total sterols content of 60–70% (Berger, Jones, & Abumweis, 2004; Verhé, Verleyen, Van Hoed, & De Greyt, 2006).

## 2 | PHYTOSTEROLS FOOD SOURCES

Vegetable oils are the major sources of PS in foods. Palm, sunflower, corn, coconut, rapeseed, and soybean oils have the highest content of total sterols; for example, rapeseed oil has a PS content ranging from 646 to 808 mg/100 g in fresh weight (FW); oil-based products like margarines have a content ranging from 130 to 540 mg/100 g FW (Normén, Ellegård, Brants, Dutta, & Andersson, 2007). Other food items such as cereals (corn, rye, wheat, barley, and oats) range from 80–90 mg/100 g FW (Ryan, Galvin, O'Connor, Maguire, & O'Brien, 2007). Additionally, cereals derivatives and nuts are great source of PS, containing the highest quantities of phytosterols compared with other foods.

Another important source of PS are fruits and berries, which are popular and highly consumed due their many beneficial nutritional properties such as their antioxidant capacity, with content ranging from 6 to 75 mg/100 g FW (Piironen, Toivo, & Lampi, 2000). PS dietary uptake varies greatly depending on the region, country and/or

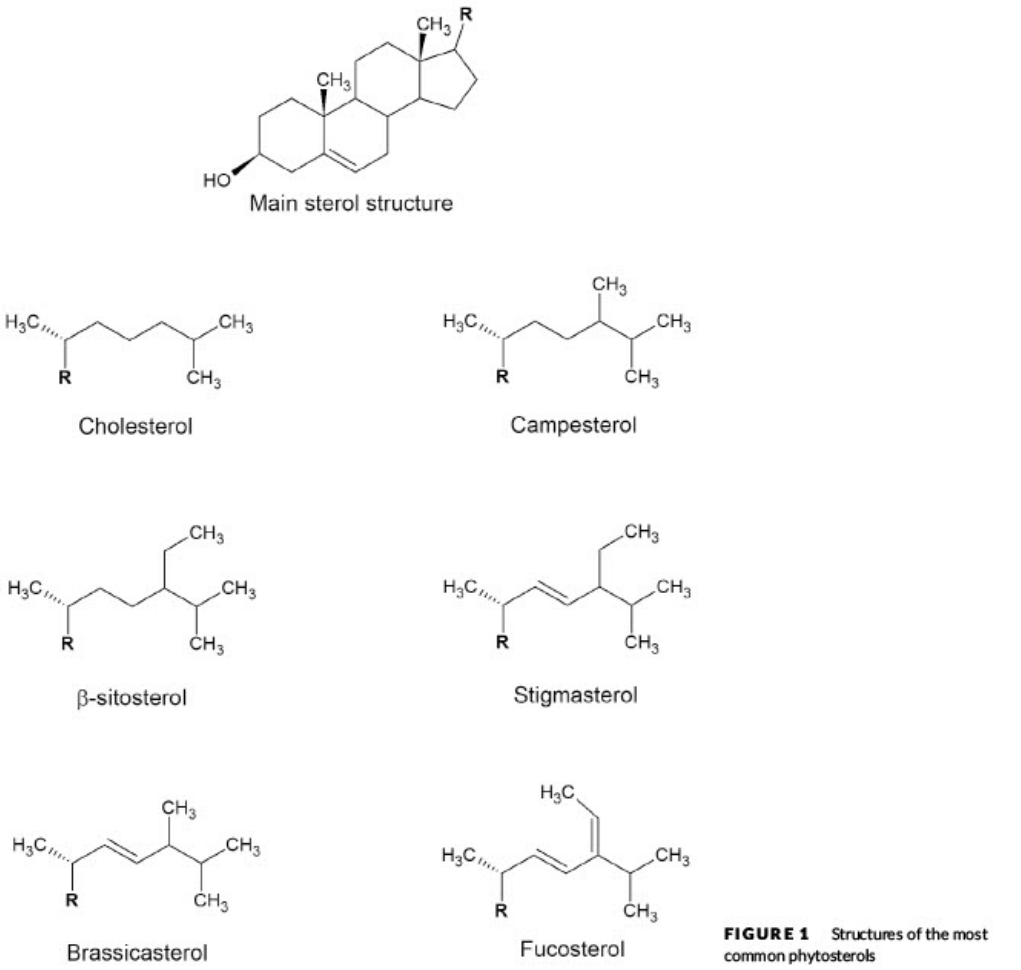


**FIGURE 1** Structures of the most common phytosterols

*Figure 7* *(cont'd)*



**TABLE 1** Phytosterol content in oils and foodstuff

| Phytosterols concentrations in different food matrices | | | | |
|---|---|---|---|---|
| **Food** | **Processing conditions** | **Phytosterol** | **Concentration** | **Reference** |
| *Oils* | | | | |
| Sunflower | N/A | Sitosterol | 233 ± 4 mg/100 g | Lin et al. (2017) |
| | | Campesterol | 34 ± 0 mg/100 g | |
| | | Stigmasterol | 32 ± 0 mg/100 g | |
| | | Sitostanol | 12 ± 0 mg/100 g | |
| Rapeseed | N/A | Sitosterol | 378 ± 13 mg/100 g | |
| | | Campesterol | 290 ± 10 mg/100 g | |
| | | Brassicasterol | 83 ± 2 mg/100 g | |
| | | Sitostanol | 59 ± 1 mg/100 g | |
| Palm | N/A | Total Phytosterols | 326–527 ppm | Mba, Dumont, and Ngadi, (2015) |
| Pine tree (tall-oil) | • >25% sterols purity (liquid emulsion)<br>• 68–75% sterols purity (powder) | Brassicasterol | • 0.01 ± 0.001 g/100 g of ingredient (>25% sterols purity)<br>• 0.03 ± 0.001 g/100 g of ingredient (68–75% sterols purity) | González-Larena et al. (2011) |
| | | Campesterol | • 1.88 ± 0.200 g/100 g of ingredient (>25% sterols purity)<br>• 5.06 ± 0.329 g/100 g of ingredient (68–75% sterols purity) | |
| | | Campestanol | • 0.26 ± 0.043 g/100 g of ingredient (>25% sterols purity)<br>• 0.85 ± 0.067 g/100 g of ingredient (68–75% sterols purity) | |
| | | Stigmasterol | • 0.16 ± 0.014 g/100 g of ingredient (>25% sterols purity)<br>• 0.58 ± 0.046 g/100 g of ingredient (68–75% sterols purity) | |
| | | β-sitosterol | • 15.97 ± 1.746 g/100 g of ingredient (>25% sterols purity)<br>• 55.94 ± 3.663 g/100 g of ingredient (68–75% sterols purity) | |
| | | Sitostanol | • 2.68 ± 0.457 g/100 g of ingredient (>25% sterols purity)<br>• 8.48 ± 0.583 g/100 g of ingredient (68–75% sterols purity) | |
| Soy | • 25% sterols purity (liquid emulsion)<br>• 68–75% sterols purity (powder) | Brassicasterol | • 0.23 ± 0.022 g/100 g of ingredient (>25% sterols purity)<br>• 0.58 ± 0.012 g/100 g of ingredient (68–75% sterols purity) | González-Larena et al. (2011) |
| | | Campesterol | • 5.91 ± 0.561 g/100 g of ingredient (>25% sterols purity)<br>• 17.02 ± 0.373 g/100 g of ingredient (68–75% sterols purity) | |
| | | Campestanol | • 0.15 ± 0.013 g/100 g of ingredient (>25% sterols purity)<br>• 0.51 ± 0.006 g/100 g of ingredient (68–75% sterols purity) | |
| | | Stigmasterol | • 6.61 ± 0.621 g/100 g of ingredient (>25% sterols purity)<br>• 17.86 ± 0.361 g/100 g of ingredient (68–75% sterols purity) | |
| | | Stigmastanol | • 0.17 ± 0.015 g/100 g of ingredient (>25% sterols purity)<br>• 0.47 ± 0.009 g/100 g of ingredient (68–75% sterols purity) | |

*Figure 7* *(cont'd)*



**TABLE 1** (Continued)

Phytosterols concentrations in different food matrices

| Food | Processing conditions | Phytosterol | Concentration | Reference |
|---|---|---|---|---|
| | | β-sitosterol | • 8.99 ± 0.920 g/100 g of ingredient (>25% sterols purity)<br>• 29.95 ± 0.673 g/100 g of ingredient (68–75% sterols purity) | |
| | | Sitostanol | • 0.32 ± 0.118 g/100 g of ingredient (>25% sterols purity)<br>• 1.63 ± 0.029 g/100 g of ingredient (68–75% sterols purity) | |
| Soybean, rapeseed, sunflower, corn oil | • 43.9% sterols purity (spray dry powder)<br>• 86–92% sterols purity (spray dry powder) | Brassicasterol | • 1.08 ± 0.129 g/100 g of ingredient (43.9% sterols purity)<br>• 1.73 ± 0.026 g/100 g of ingredient (86–92% sterols purity) | |
| *Oils* | | | | |
| Soybean, rapeseed, sunflower, corn oil | • 43.9% sterols purity (spray dry powder)<br>• 86–92% sterols purity (spray dry powder) | Campesterol | • 7.71 ± 0.998 g/100 g of ingredient (43.9% sterols purity)<br>• 10.87 ± 0.181 g/100 g of ingredient (86–92% sterols purity) | González-Larena et al. (2011) |
| | | Campestanol | • 0.40 ± 0.053 g/100 g of ingredient (43.9% sterols purity)<br>• 0.90 ± 0.020 g/100 g of ingredient (86–92% sterols purity) | |
| | | Stigmasterol | • 0.61 ± 0.040 g/100 g of ingredient (43.9% sterols purity)<br>• 0.93 ± 0.020 g/100 g of ingredient (86–92% sterols purity) | |
| | | β-sitosterol | • 31.17 ± 4.732 g/100 g of ingredient (43.9% sterols purity)<br>• 63.51 ± 1.627 g/100 g of ingredient (86–92% sterols purity) | |
| | | Sitostanol | • 3.97 ± 0.542 g/100 g of ingredient (43.9% sterols purity)<br>• 8.47 ± 0.175 g/100 g of ingredient (86–92% sterols purity) | |
| Sunflower, tall-oils | 12% sterols purity (liquid emulsion) | Campesterol | 0.95 ± 0.131 g/100 g of ingredient | |
| | | Campestanol | 0.15 ± 0.023 g/100 g of ingredient | |
| | | Stigmasterol | 0.11 ± 0.012 g/100 g of ingredient | |
| | | β-sitosterol | 10.42 ± 1.954 g/100 g of ingredient | |
| | | Sitostanol | 1.43 ± 0.147 g/100 g of ingredient | |
| High oleic sunflower, tall-oils | 30% sterols purity (oil paste) | Brassicasterol | 0.01 ± 0.001 g/100 g of ingredient | |
| | | Campesterol | 2.34 ± 0.114 g/100 g of ingredient | |
| | | Campestanol | 0.37 ± 0.016 g/100 g of ingredient | |
| | | Stigmasterol | 0.27 ± 0.005 g/100 g of ingredient | |
| | | β-sitosterol | 25.40 ± 1.608 g/100 g of ingredient | |
| | | Sitostanol | 3.66 ± 0.214 g/100 g of ingredient | |
| Peanut | • Control<br>• Heating (microwave for 1 min)<br>• Heating (microwave for 2 min)<br>• Heating (microwave for 3 min) | Total Phytosterols | • 273.55 ± 2.51 mg/100 g (control)<br>• 298.49 ± 3.26 mg/100 g (microwave for 1 min)<br>• 310.33 ± 5.21 mg/100 g (microwave for 2 min)<br>• 325.13 ± 6.76 mg/100 g (microwave for 3 min) | Hu et al. (2018) |

*Figure 7* *(cont'd)*



**TABLE 1** (Continued)

Phytosterols concentrations in different food matrices

| Food | Processing conditions | Phytosterol | Concentration | Reference |
|---|---|---|---|---|
| | • Heating (microwave for 4 min)<br>• Heating (microwave for 5 min) | | • 339.16 ± 3.73 mg/100 g (microwave for 4 min)<br>• 363.35 ± 4.22 mg/100 g (microwave for 5 min) | |
| | N/A | Campesterol | 0.397 mg/g | Islam et al. (2017) |
| | | Stigmasterol | 0.224 mg/g | |
| | | β-sitosterol | 1.385 mg/g | |
| | | Avenasterol | 0.163 mg/g | |
| Maize | N/A | β-sitosterol | 4,794 mg/kg fresh weight | Plumb, Rhodes, Lampi, Buchgraber, and Kroon (2011) |
| | | Campesterol | 1,498 mg/kg fresh weight | |
| | | Stigmasterol | 516 mg/kg fresh weight | |
| | | Δ-5-Avenasterol | 450 mg/kg fresh weight | |
| | | Δ-7-Avenasterol | ND | |
| | | Brassicasterol | 48 mg/kg fresh weight | |
| | | Campestanol | 116 mg/kg fresh weight | |
| | | Sitostanol | 212 mg/kg fresh weight | |
| Rape | | β-sitosterol | 3,563 mg/kg fresh weight | |
| | | Campesterol | 2,341 mg/kg fresh weight | |
| | | Stigmasterol | 75 mg/kg fresh weight | |
| | | Δ-5-Avenasterol | 250 mg/kg fresh weight | |
| | | Δ-7-Avenasterol | ND | |
| | | Brassicasterol | 767 mg/kg fresh weight | |
| | | Campestanol | 19 mg/kg fresh weight | |
| | | Sitostanol | 29 mg/kg fresh weight | |
| Sunflower | | β-sitosterol | 2,169 mg/kg fresh weight | |
| | | Campesterol | 336 mg/kg fresh weight | |
| | | Stigmasterol | 332 mg/kg fresh weight | |
| | | Δ-5-Avenasterol | 232 mg/kg fresh weight | |
| | | Δ-7-Avenasterol | 249 mg/kg fresh weight | |
| | | Brassicasterol | 21 mg/kg fresh weight | |
| | | Campestanol | 12 mg/kg fresh weight | |
| | | Sitostanol | 51 mg/kg fresh weight | |
| Groundnut | | β-sitosterol | 2,728 mg/kg fresh weight | |
| | | Campesterol | 308 mg/kg fresh weight | |
| | | Stigmasterol | 231 mg/kg fresh weight | |
| | | Δ-5-Avenasterol | 187 mg/kg fresh weight | |
| | | Δ-7-Avenasterol | ND | |
| | | Brassicasterol | 36 mg/kg fresh weight | |
| | | Campestanol | 11 mg/kg fresh weight | |
| | | Sitostanol | 27 mg/kg fresh weight | |
| Soya | | β-sitosterol | 1,438 mg/kg fresh weight | |
| | | Campesterol | 669 mg/kg fresh weight | |
| | | Stigmasterol | 559 mg/kg fresh weight | |
| | | Δ-5-Avenasterol | 90 mg/kg fresh weight | |
| | | Δ-7-Avenasterol | 49 mg/kg fresh weight | |
| | | Brassicasterol | 17 mg/kg fresh weight | |

*Figure 7* *(cont'd)*



**TABLE 1** (Continued)

Phytosterols concentrations in different food matrices

| Food | Processing conditions | Phytosterol | Concentration | Reference |
|---|---|---|---|---|
| | | Campestanol | 26 mg/kg fresh weight | |
| | | Sitostanol | ND | |
| Olive | | β-sitosterol | 1,663 mg/kg fresh weight | |
| | | Campesterol | 55 mg/kg fresh weight | |
| | | Stigmasterol | 57 mg/kg fresh weight | |
| | | Δ-5-Avenasterol | 342 mg/kg fresh weight | |
| | | Δ-7-Avenasterol | 3 mg/kg fresh weight | |
| | | Brassicasterol | 11 mg/kg fresh weight | |
| | | Campestanol | 4 mg/kg fresh weight | |
| | | Sitostanol | 20 mg/kg fresh weight | |
| Hazel | | β-sitosterol | 1,256 mg/kg fresh weight | |
| | | Campesterol | 85 mg/kg fresh weight | |
| | | Stigmasterol | 18 mg/kg fresh weight | |
| | | Δ-5-Avenasterol | 78 mg/kg fresh weight | |
| | | Δ-7-Avenasterol | 14 mg/kg fresh weight | |
| | | Brassicasterol | ND | |
| | | Campestanol | 3 mg/kg fresh weight | |
| | | Sitostanol | NS | |
| Soybean | • Control<br>• Hydrogenated<br>• Expeller pressed<br>• Expeller pressed low Linolenic<br>• Acid | Campesterol | • 33.5 ± 1.2 mg/100 g (control)<br>• 40.8 ± 1.3 mg/100 g (hydrogenated)<br>• 39.8 ± 0.85 mg/100 g (expeller pressed)<br>• 52.3 ± 1.6 mg/100 g (expeller pressed low linoleic acid) | Winkler, Warner, and Glynn (2007) |
| | | Stigmasterol | • 57.0 ± 1.6 mg/100 g (control)<br>• 66.0 ± 1.9 mg/100 g (hydrogenated)<br>• 61.4 ± 0.9 mg/100 g (expeller pressed)<br>• 47.9 ± 1.3 mg/100 g (expeller pressed low linoleic acid) | |
| | | β-sitosterol | • 33.5 ± 1.2 mg/100 g (control)<br>• 40.8 ± 1.3 mg/100 g (hydrogenated)<br>• 39.8 ± 0.85 mg/100 g (expeller pressed)<br>• 52.3 ± 1.6 mg/100 g (expeller pressed low linoleic acid) | |
| | | Sitostanol | • Tr (control)<br>• Tr (hydrogenated)<br>• Tr (expeller pressed)<br>• Tr (expeller pressed low linoleic acid) | |
| | | Avenasterol | • 10.7 ± 3.0 mg/100 g (control)<br>• 12.3 ± 3.4 mg/100 g (hydrogenated)<br>• 12.0 ± 1.1 mg/100 g (expeller pressed)<br>• 13.0 ± 1.3 mg/100 g (expeller pressed low linoleic acid) | |
| | | Others | • Tr (control)<br>• Tr (hydrogenated)<br>• Tr (expeller pressed)<br>• Tr (expeller pressed low linoleic acid) | |
| Sunflower | High oleic content | Campesterol | 11.7 ± 1.7 mg/100 g | |
| | | Stigmasterol | 24.3 ± 2.1 mg/100 g | |
| | | β-sitosterol | 229.5 ± 7.8 mg/100 g | |
| | | Sitostanol | Tr | |

*Figure 7* *(cont'd)*



**TABLE 1** (Continued)

| Phytosterols concentrations in different food matrices | | | | |
|---|---|---|---|---|
| **Food** | **Processing conditions** | **Phytosterol** | **Concentration** | **Reference** |
| | | Avenasterol | 15.7 ± 0.5 mg/100 g | |
| | | Others | 115.7 ± 6.4 mg/100 g | |
| Corn | N/A | Campesterol | 98.6 ± 3.2 mg/100 g | |
| | | Stigmasterol | 64.3 ± 2.5 mg/100 g | |
| | | β-sitosterol | 611.8 ± 19.9 mg/100 g | |
| | | Sitostanol | 29.8 ± 1.4 mg/100 g | |
| | | Avenasterol | 40.1 ± 1.3 mg/100 g | |
| | | Others | 1.0 ± 0.08 mg/100 g | |
| Maize | N/A | Campesterol | 42 mg/100 g food product | Johnsson and Dutta, (2006a) |
| | | Stigmasterol | 37 mg/100 g food product | |
| | | Sitosterol | 179 mg/100 g food product | |
| Peanut | | Campesterol | 36 mg/100 g food product | |
| | | Stigmasterol | 31 mg/100 g food product | |
| | | Sitosterol | 143 mg/100 g food product | |
| Olive | | Campesterol | 18 mg/100 g food product | |
| | | Stigmasterol | 6 mg/100 g food product | |
| | | Sitosterol | 78 mg/100 g food product | |
| *Dairy* | | | | |
| Brick margarine | N/A | Sitosterol | 158 ± 8 mg/100 g | Lin et al. (2017) |
| | | Campesterol | 103 ± 5 mg/100 g | |
| | | Stigmasterol | 7 ± 0 mg/100 g | |
| | | Sitostanol | 19 ± 1 mg/100 g | |
| | | Brassicasterol | 24 ± 1 mg/100 g | |
| Liquid margarine | N/A | Sitosterol | 196 ± 6 mg/100 g | |
| | | Campesterol | 92 ± 1 mg/100 g | |
| | | Stigmasterol | 17 ± 1 mg/100 g | |
| | | Sitostanol | 16 ± 1 mg/100 g | |
| | | Brassicasterol | 16 ± 0 mg/100 g | |
| 35% spread | Storage (18 weeks) | Brassicasterol | 0.02 ± 0.00 g/100 g spread | Nieminen, Laakso, Kuusisto, Niemelä, and Laitinen (2016) |
| | | Campesterol | 0.21 ± 0.01 g/100 g spread | |
| | | Campestanol | 1.06 ± 0.00 g/100 g spread | |
| | | Sitosterol | 0.24 ± 0.00 g/100 g spread | |
| | | Sitostanol | 5.55 ± 0.01 g/100 g spread | |
| 60% spread | Storage (18 weeks) | Brassicasterol | 0.04 ± 0.00 g/100 g spread | |
| | | Campesterol | 0.26 ± 0.01 g/100 g spread | |
| | | Campestanol | 0.90 ± 0.01 g/100 g spread | |
| | | Sitosterol | 0.35 ± 0.01 g/100 g spread | |
| | | Sitostanol | 5.66 ± 0.02 g/100 g spread | |
| Maize yogurt | Fermented | β-sitosterol | 0.062 ± 0.005 g/kg | Descalzo et al. (2018) |
| | | Campesterol + Stigmasterol | 0.022 ± 0.003 g/kg | |
| Milk | N/A | Total phytosterols | 0.156 ± 0.00 g/100 g | Srigley and Haile (2015) |
| *Eggs* | | | | |
| Eggs | N/A | Total phytosterols | 20.7 mg sterols/100 g ingredient | Menéndez-Carreño, Knol, and Janssen (2016) |

*Figure 7* *(cont'd)*



**TABLE 1** (Continued)

Phytosterols concentrations in different food matrices

| Food | Processing conditions | Phytosterol | Concentration | Reference |
|---|---|---|---|---|
| *Meat and Poultry* | | | | |
| Meat (steak, roast beef, stew, chicken, pork, and minced meat) | N/A | Total phytosterols | 3.47–14.9 mg sterols/100 g ingredient | Menéndez-Carreño et al. (2016) |
| *Seafood* | | | | |
| Fish (salmon, shallow-fried cod, microwaved cod, and fish sticks) | N/A | Total phytosterols | 2.01–51.9 mg sterols/100 g ingredient | Menéndez-Carreño et al. (2016) |
| *Others* | | | | |
| Margarine | • Control<br>• Storage (18 weeks @ 4°C)<br>• Storage (18 weeks @ 20°C) | Brassicasterol | • 0.82 ± 0.18 g/100 g spread (control)<br>• 0.71 ± 0.06 g/100 g spread (18 weeks @ 4°C)<br>• 0.58 ± 0.05 g/100 g spread (18 weeks @ 20°C) | Rudzińska, Przybylski, and Wąsowicz, (2014) |
| | | Campesterol | • 2.95 ± 0.23 g/100 g spread (control)<br>• 2.12 ± 0.18 g/100 g spread (18 weeks @ 4°C)<br>• 2.04 ± 0.18 g/100 g spread (18 weeks @ 20°C) | |
| Vegetable and potato (onion, cabbage, green beans, and potato) | N/A | Total phytosterols | 7.3–37.5 mg sterols/100 g ingredient | Menéndez-Carreño, Knol, and Janssen (2016) |
| Light mayonnaise | N/A | Total | 0.686 ± 0.01 g/100 g | Srigley and Haile (2015) |
| Spread/margarine-1 | | Phytosterols | 0.788 ± 0.01 g/100 g | |
| Spread/margarine-2 | | | 0.753 ± 0.01 g/100 g | |
| Spread/margarine-3 | | | 2.88 ± 0.02 g/100 g | |
| Spread/margarine-4 | | | 3.82 ± 0.04 g/100 g | |
| Orange juice | | | 0.423 ± 0.01 g/100 g | |
| Protein shake-1 | | | 0.529 ± 0.02 g/100 g | |
| Protein shake-2 | | | 4.86 ± 0.60 g/100 g | |
| Instant coffee | | | 6.96 ± 0.69 g/100 g | |
| Dietary chew-1 | | | 7.73 ± 0.11 g/100 g | |
| Dietary chew-2 | | | 9.61 ± 0.09 g/100 g | |
| *Others* | | | | |
| Margarine | • Control<br>• Storage (18 weeks @ 4°C)<br>• Storage (18 weeks @ 20°C) | Campestanol | • 12.7 ± 1.02 g/100 g spread (control)<br>• 11.3 ± 1.05 g/100 g spread (18 weeks @ 4°C)<br>• 8.29 ± 0.76 g/100 g spread (18 weeks @ 20°C) | Rudzińska et al. (2014) |
| | | Sitosterol | • 3.62 ± 0.26 g/100 g spread (control)<br>• 2.95 ± 0.22 g/100 g spread (18 weeks @ 4°C)<br>• 2.23 ± 0.20 g/100 g spread (18 weeks @ 20°C) | |
| | | Sitostanol | • 58.1 ± 4.31 g/100 g spread (control)<br>• 45.1 ± 4.05 g/100 g spread (18 weeks @ 4°C)<br>• 40.9 ± 3.73 g/100 g spread (18 weeks @ 20°C) | |

*Figure 7* *(cont'd)*



**TABLE 1** (Continued)

Phytosterols concentrations in different food matrices

| Food | Processing conditions | Phytosterol | Concentration | Reference |
|---|---|---|---|---|
| | | Avenasterol | • 0.68 ± 0.06 g/100 g spread (control)<br>• 0.56 ± 0.05 g/100 g spread (18 weeks @ 4°C)<br>• 0.56 ± 0.05 g/100 g spread (18 weeks @ 20°C) | |
| | N/A | Brassicasterol | 185.39 mg/100 g | Menéndez-Carreño et al. (2016) |
| | | Campesterol | 1,030.19 mg/100 g | |
| | | Stigmasterol | 37.16 mg/100 g | |
| | | β-sitosterol | 5,169.30 mg/100 g | |
| | • From the Netherlands:<br>○ AH<br>○ Brio<br>○ Low trans 1<br>○ Low trans 2<br>○ Wajang<br>○ Blue band<br>○ Bona<br>○ Het Volle pond<br>○ Ruitjes<br>○ Zeeuws Meisje<br>○ Golden Regen<br>○ Halvarine blue (40% fat)<br>○ Halvarine Golden Regen (40% fat)<br>○ Halvarine gouda's Glorie (40% fat)<br>○ Halvarine light (40% fat)<br>○ Halvarine low 35% (40% fat)<br>○ Low fat spread (40% fat)<br>○ Becel 70% (70% fat)<br>○ Dieetella (70% fat)<br>• From Sweden:<br>○ Det goda<br>○ Flora<br>○ Linnea<br>○ Melba<br>○ Runda<br>○ Ädel<br>○ Milda<br>○ Treess<br>○ Vitaqell (80% fat, poly)<br>○ Lätt & Lagom (40% fat)<br>○ Lätta (40% fat)<br>○ Nytta (40% fat, poly)<br>○ Carlshamns (liquid 80% fat)<br>○ Milda (liquid 80% fat) | Campesterol | • From the Netherlands:<br>○ 47 mg/100 g (AH)<br>○ 54 mg/100 g (brio)<br>○ 36 mg/100 g (low trans 1)<br>○ 35 mg/100 g (low trans 2)<br>○ 38 mg/100 g (Wajang)<br>○ 28 mg/100 g (blue band)<br>○ 56 mg/100 g (Bona)<br>○ 45 mg/100 g (Het Volle pond)<br>○ 74 mg/100 g (Ruitjes)<br>○ 27 mg/100 g (Zeeuws Meije)<br>○ 29 mg/100 g (Golden Regen)<br>○ 23 mg/100 g (Halvarine blue, 40% fat)<br>○ 24 mg/100 g (Halvarine Golden Regen, 40% fat)<br>○ 24 mg/100 g (Halvarine gouda's Glorie, 40% fat)<br>○ 12 mg/100 g (Halvarine light, 40% fat)<br>○ 17 mg/100 g (Halvarine low 35%, 40% fat)<br>○ 24 mg/100 g (low fat spread, 40% fat)<br>○ 42 mg/100 g (Lätt & Lagon, 40% fat)<br>○ 38 mg/100 g (Lätta, 40% fat)<br>○ 134 mg/100 g (Nytta, 40% fat, poly)<br>○ 29 mg/100 g (Becel 70%, 70% fat)<br>○ 31 mg/100 g (Dieetella, 70% fat)<br>• From Sweden:<br>○ 66 mg/100 g (Det goda)<br>○ 104 mg/100 g (Flora)<br>○ 93 mg/100 g (Linnea)<br>○ 84 mg/100 g (Melba)<br>○ 90 mg/100 g (Runda)<br>○ 66 mg/100 g (Ädel)<br>○ 86 mg/100 g (Milda)<br>○ 62 mg/100 g (Tre ess)<br>○ 170 mg/100 g (Vitaqell, 80% fat, poly)<br>○ 42 mg/100 g (Lätt & Lagom (40% fat)<br>○ 38 mg/100 g (Lätta (40% fat)<br>○ 134 mg/100 g (Nytta (40% fat, poly)<br>○ 230 mg/100 g (Carlshamns, liquid 80% fat)<br>○ 172 mg/100 g (Milda, liquid 80% fat)<br>○ 238 mg/100 g (Bytta, liquid 80% fat)<br>○ 75 mg/100 g (Vigör, liquid 80% fat) | |

*Figure 7* *(cont'd)*



**TABLE 1** (Continued)

Phytosterols concentrations in different food matrices

| Food | Processing conditions | Phytosterol | Concentration | Reference |
|---|---|---|---|---|
| | ◦ Bytta (liquid 80% fat)<br>◦ Vigör (liquid 80% fat)<br>◦ Bregott (liquid with butter 80% fat) | | ◦ 68 mg/100 g (Bregott, liquid with butter 80% fat) | |
| | | Campestanol | • From the Netherlands:<br>◦ 2.2 mg/100 g (AH)<br>◦ 0.0 mg/100 g (brio)<br>◦ 0.0 mg/100 g (low trans 1)<br>◦ 0.0 mg/100 g (low trans 2)<br>◦ 0.0 mg/100 g (Wajang)<br>◦ 0.0 mg/100 g (blue band)<br>◦ 2.1 mg/100 g (Bona)<br>◦ 1.9 mg/100 g (Het Volle pond)<br>◦ 0.0 mg/100 g (Ruitjes)<br>◦ 1.5 mg/100 g (Zeeuws Meije)<br>◦ 0.0 mg/100 g (Golden Regen)<br>◦ 0.0 mg/100 g (Halvarine blue, 40% fat)<br>◦ 1.4 mg/100 g (Halvarine Golden Regen, 40% fat)<br>◦ 0.0 mg/100 g (Halvarine gouda's Glorie, 40% fat)<br>◦ 0.0 mg/100 g (Halvarine light, 40% fat)<br>◦ 0.0 mg/100 g (Halvarine low 35%, 40% fat)<br>◦ 0.0 mg/100 g (low fat spread, 40% fat)<br>◦ 3.5 mg/100 g (Becel 70%, 70% fat)<br>◦ 4.6 mg/100 g (Dieetella, 70% fat)<br>◦ 0.0 mg/100 g (Carlshamns, liquid 80% fat)<br>◦ 0.0 mg/100 g (Milda, liquid 80% fat)<br>◦ 0.0 mg/100 g (Nytta, liquid 80% fat)<br>◦ 3.1 mg/100 g (Vigör, liquid 80% fat)<br>◦ 0.0 mg/100 g (Bregott, with butter 80% fat)<br>• From Sweden:<br>◦ 0.0 mg/100 g (Det goda)<br>◦ 0.0 mg/100 g (Flora)<br>◦ 0.0 mg/100 g (Linnea)<br>◦ 0.0 mg/100 g (Melba)<br>◦ 0.0 mg/100 g (Runda)<br>◦ 0.0 mg/100 g (Ädel)<br>◦ 0.0 mg/100 g (Milda)<br>◦ 0.0 mg/100 g (Tre ess)<br>◦ 0.0 mg/100 g (Vitaqell, 80% fat, poly)<br>◦ 0.0 mg/100 g (Lätt & Lagom (40% fat)<br>◦ 0.0 mg/100 g (Lätta (40% fat)<br>◦ 0.0 mg/100 g (Nytta (40% fat, poly)<br>◦ 0.0 mg/100 g (Carlshamns, liquid 80% fat)<br>◦ 0.0 mg/100 g (Milda, liquid 80% fat)<br>◦ 0.0 mg/100 g (Bytta, liquid 80% fat)<br>◦ 3.1 mg/100 g (Vigör, liquid 80% fat)<br>◦ 0.0 mg/100 g (Bregott, liquid with butter 80% fat) | |

*Figure 7* *(cont'd)*



**TABLE 1** (Continued)

Phytosterols concentrations in different food matrices

| Food | Processing conditions | Phytosterol | Concentration | Reference |
|---|---|---|---|---|
| | | Stigmasterol | • From the Netherlands:<br>◦ 41 mg/100 g (AH)<br>◦ 25 mg/100 g (brio)<br>◦ 30 mg/100 g (low trans 1)<br>◦ 29 mg/100 g (low trans 2)<br>◦ 33 mg/100 g (Wajang)<br>◦ 23 mg/100 g (blue band)<br>◦ 46 mg/100 g (Bona)<br>◦ 38 mg/100 g (Het Volle pond)<br>◦ 2 mg/100 g (Ruitjes)<br>◦ 20 mg/100 g (Zeeuws Meije)<br>◦ 19 mg/100 g (Golden Regen)<br>◦ 20 mg/100 g (Halvarine blue, 40% fat)<br>◦ 17 mg/100 g (Halvarine Golden Regen, 40% fat)<br>◦ 21 mg/100 g (Halvarine gouda's Glorie, 40% fat)<br>◦ 11 mg/100 g (Halvarine light, 40% fat)<br>◦ 14 mg/100 g (Halvarine low 35%, 40% fat)<br>◦ 18 mg/100 g (low fat spread, 40% fat)<br>◦ 26 mg/100 g (Becel 70%, 70% fat)<br>◦ 32 mg/100 g (Dieetella, 70% fat)<br>• From Sweden:<br>◦ 5 mg/100 g (Det goda)<br>◦ 7 mg/100 g (Flora)<br>◦ 6 mg/100 g (Linnea)<br>◦ 6 mg/100 g (Melba)<br>◦ 6 mg/100 g (Runda)<br>◦ 6 mg/100 g (Ädel)<br>◦ 8 mg/100 g (Milda)<br>◦ 8 mg/100 g (Tre ess)<br>◦ 53 mg/100 g (Vitaqell, 80% fat, poly)<br>◦ 0.0 mg/100 g (Lätt & Lagom (40% fat)<br>◦ 4 mg/100 g (Lätta (40% fat)<br>◦ 5 mg/100 g (Nytta (40% fat, poly)<br>◦ 5 mg/100 g (Carlshamns, liquid 80% fat)<br>◦ 3 mg/100 g (Milda, liquid 80% fat)<br>◦ 4 mg/100 g (Bytta, liquid 80% fat)<br>◦ 21 mg/100 g (Vigör, liquid 80% fat)<br>◦ 5 mg/100 g (Bregott, liquid with butter 80% fat) | |
| | | Sitosterol | • From the Netherlands:<br>◦ 120 mg/100 g (AH)<br>◦ 128 mg/100 g (brio)<br>◦ 95 mg/100 g (low trans 1)<br>◦ 83 mg/100 g (low trans 2)<br>◦ 94 mg/100 g (Wajang)<br>◦ 77 mg/100 g (blue band)<br>◦ 138 mg/100 g (Bona)<br>◦ 121 mg/100 g (Het Volle pond)<br>◦ 100 mg/100 g (Ruitjes)<br>◦ 84 mg/100 g (Zeeuws Meije)<br>◦ 67 mg/100 g (Golden Regen)<br>◦ 59 mg/100 g (Halvarine blue, 40% fat) | |

*Figure 7* *(cont'd)*



**TABLE 1** (Continued)

Phytosterols concentrations in different food matrices

| Food | Processing conditions | Phytosterol | Concentration | Reference |
|---|---|---|---|---|
| | | | ◦ 49 mg/100 g (Halvarine Golden Regen, 40% fat)<br>◦ 64 mg/100 g (Halvarine gouda's Glorie, 40% fat)<br>◦ 76 mg/100 g (Halvarine light, 40% fat)<br>◦ 100.0 mg/100 g (Halvarine low 35%, 40% fat)<br>◦ 63 mg/100 g (low fat spread, 40% fat)<br>◦ 176 mg/100 g (Becel 70%, 70% fat)<br>◦ 218 mg/100 g (Dieetella, 70% fat)<br>• From Sweden:<br>◦ 87 mg/100 g (Det goda)<br>◦ 132 mg/100 g (Flora)<br>◦ 123 mg/100 g (Linnea)<br>◦ 108 mg/100 g (Melba)<br>◦ 123 mg/100 g (Runda)<br>◦ 89 mg/100 g (Ädel)<br>◦ 135 mg/100 g (Milda)<br>◦ 96 mg/100 g (Tre ess)<br>◦ 469 mg/100 g (Vitaqell, 80% fat, poly)<br>◦ 63 mg/100 g (Lätt & Lagom, 40% fat)<br>◦ 57 mg/100 g (Lätta (40% fat)<br>◦ 165 mg/100 g (Nytta (40% fat, poly)<br>◦ 288 mg/100 g (Carlshamns, liquid 80% fat)<br>◦ 225 mg/100 g (Milda, liquid 80% fat)<br>◦ 274 mg/100 g (Bytta, liquid 80% fat)<br>◦ 215 mg/100 g (Vigör, liquid 80% fat)<br>◦ 77 mg/100 g (Bregott, liquid with butter 80% fat) | |
| | | Sitostanol | • From the Netherlands:<br>◦ 3.1 mg/100 g (AH)<br>◦ 2.8 mg/100 g (brio)<br>◦ 0.0 mg/100 g (low trans 1)<br>◦ 2.1 mg/100 g (low trans 2)<br>◦ 0.0 mg/100 g (Wajang)<br>◦ 2.6 mg/100 g (blue band)<br>◦ 4.2 mg/100 g (Bona)<br>◦ 3.6 mg/100 g (Het Volle pond)<br>◦ 0.0 mg/100 g (Ruitjes)<br>◦ 1.9 mg/100 g (Zeeuws Meije)<br>◦ 1.3 mg/100 g (Golden Regen)<br>◦ 0.0 mg/100 g (Halvarine blue, 40% fat)<br>◦ 1.2 mg/100 g (Halvarine Golden Regen, 40% fat)<br>◦ 0.0 mg/100 g (Halvarine gouda's Glorie, 40% fat)<br>◦ 1.9 mg/100 g (Halvarine light, 40% fat)<br>◦ 0.0 mg/100 g (Halvarine low 35%, 40% fat)<br>◦ 2.2 mg/100 g (low fat spread, 40% fat)<br>◦ 1.6 mg/100 g (Becel 70%, 70% fat)<br>◦ 2.1 mg/100 g (Dieetella, 70% fat) | |

*Figure 7* *(cont'd)*



**TABLE 1** (Continued)

Phytosterols concentrations in different food matrices

| Food | Processing conditions | Phytosterol | Concentration | Reference |
|---|---|---|---|---|
| | | | • From Sweden:<br>○ 0.0 mg/100 g (Det goda)<br>○ 0.0 mg/100 g (Flora)<br>○ 0.0 mg/100 g (Linnea)<br>○ 0.0 mg/100 g (Melba)<br>○ 0.0 mg/100 g (Runda)<br>○ 4.3 mg/100 g (Ädel)<br>○ 0.0 mg/100 g (Milda)<br>○ 0.0 mg/100 g (Tre ess)<br>○ 19 mg/100 g (Vitaqell, 80% fat, poly)<br>○ 0.0 mg/100 g (Lätt & Lagom, 40% fat)<br>○ 0.0 mg/100 g (Lätta, 40% fat)<br>○ 0.0 mg/100 g (Nytta, 40% fat, poly)<br>○ 0.0 mg/100 g (Carlshamns, liquid 80% fat)<br>○ 0.0 mg/100 g (Milda, liquid 80% fat)<br>○ 0.0 mg/100 g (Bytta, liquid 80% fat)<br>○ 2.8 mg/100 g (Vigör, liquid 80% fat)<br>○ 0.0 mg/100 g (Bregott, liquid with butter 80% fat) | |
| | | Brassicasterol | • From Netherlands:<br>○ 2.1 mg/100 g (AH)<br>○ 0.0 mg/100 g (brio)<br>○ 0.0 mg/100 g (low trans 1)<br>○ 0.0 mg/100 g (low trans 2)<br>○ 11 mg/100 g (Wajang)<br>○ 0.0 mg/100 g (blue band)<br>○ 3.2 mg/100 g (Bona)<br>○ 5.9 mg/100 g (Het Volle pond)<br>○ 1.5 mg/100 g (Ruitjes)<br>○ 7.3 mg/100 g (Zeeuws Meije)<br>○ 4 mg/100 g (Golden Regen)<br>○ 0.0 mg/100 g (Halvarine blue, 40% fat)<br>○ 2 mg/100 g (Halvarine Golden Regen, 40% fat)<br>○ 0.0 mg/100 g (Halvarine gouda's Glorie, 40% fat)<br>○ 0.0 mg/100 g (Halvarine light, 40% fat)<br>○ 0.0 mg/100 g (Halvarine low 35%, 40% fat)<br>○ 5.4 mg/100 g (low fat spread, 40% fat)<br>○ 3.3 mg/100 g (Becel 70%, 70% fat)<br>○ 1.9 mg/100 g (Dieetella, 70% fat)<br>• From Sweden:<br>○ 15 mg/100 g (Det goda)<br>○ ND (Flora)<br>○ 22 mg/100 g (Linnea)<br>○ 21 mg/100 g (Melba)<br>○ 8.0 mg/100 g (Runda)<br>○ 9.1 mg/100 g (Ädel)<br>○ 20 mg/100 g (Milda)<br>○ 14 mg/100 g (Tre ess)<br>○ 0.0 mg/100 g (Vitaqell, 80% fat, poly)<br>○ 10.5 mg/100 g (Lätt & Lagom, 40% fat)<br>○ 9.4 mg/100 g (Lätta, 40% fat)<br>○ ND (Nytta, 40% fat, poly) | |

*Figure 7* *(cont'd)*



**TABLE 1** (Continued)

Phytosterols concentrations in different food matrices

| Food | Processing conditions | Phytosterol | Concentration | Reference |
|---|---|---|---|---|
| | | | ○ ND (Carlshamns, liquid 80% fat)<br>○ 46 mg/100 g (Milda, liquid 80% fat)<br>○ 58 mg/100 g (Bytta, liquid 80% fat)<br>○ 8.9 mg/100 g (Vigör, liquid 80% fat)<br>○ 19 mg/100 g (Bregott, liquid with butter 80% fat) | |
| | | Δ-5-Avenasterol | • From Netherlands:<br>○ 4.5 mg/100 g (AH)<br>○ 9 mg/100 g (brio)<br>○ 5.9 mg/100 g (low trans 1)<br>○ 6.2 mg/100 g (low trans 2)<br>○ 6.5 mg/100 g (Wajang)<br>○ 0.0 mg/100 g (blue band)<br>○ 6.9 mg/100 g (Bona)<br>○ 4.6 mg/100 g (Het Volle pond)<br>○ 5.8 mg/100 g (Ruitjes)<br>○ 6.2 mg/100 g (Zeeuws Meije)<br>○ 4.8 mg/100 g (Golden Regen)<br>○ 3 mg/100 g (Halvarine blue, 40% fat)<br>○ 2.4 mg/100 g (Halvarine Golden Regen, 40% fat)<br>○ 3 mg/100 g (Halvarine gouda's Glorie, 40% fat)<br>○ 5.5 mg/100 g (Halvarine light, 40% fat)<br>○ 9.1 mg/100 g (Halvarine low 35%, 40% fat)<br>○ 2.3 mg/100 g (low fat spread, 40% fat)<br>○ 14.8 mg/100 g (Becel 70%, 70% fat)<br>○ 16.3 mg/100 g (Dieetella, 70% fat)<br>• From Sweden:<br>○ 7.8 mg/100 g (Det goda)<br>○ ND (Flora)<br>○ 15 mg/100 g (Linnea)<br>○ 10.9 mg/100 g (Melba)<br>○ 10.2 mg/100 g (Runda)<br>○ 6.9 mg/100 g (Ädel)<br>○ 12.5 mg/100 g (Milda)<br>○ 11.9 mg/100 g (Tre ess)<br>○ 54.2 mg/100 g (Vitaqell, 80% fat, poly)<br>○ 0.0 mg/100 g (Lätt & Lagom, 40% fat)<br>○ 5.1 mg/100 g (Lätta, 40% fat)<br>○ ND (Nytta, 40% fat, poly)<br>○ ND (Carlshamns, liquid 80% fat)<br>○ 16.6 mg/100 g (Milda, liquid 80% fat)<br>○ 24.5 mg/100 g (Bytta, liquid 80% fat)<br>○ 11.3 mg/100 g (Vigör, liquid 80% fat)<br>○ 7 mg/100 g (Bregott, liquid with butter 80% fat) | |
| Fat | • From Netherlands:<br>○ Baking fat<br>○ Cooking fat 1<br>○ Cooking fat 2<br>○ Frying fat 1<br>○ Frying fat 2<br>• From Sweden:<br>○ Coconut fat | Campesterol | • From Netherlands:<br>○ 66 mg/100 g (baking fat)<br>○ 37 mg/100 g (cooking fat 1)<br>○ 33 mg/100 g (cooking fat 2)<br>○ 147 mg/100 g (frying fat 1)<br>○ 49 mg/100 g (frying fat 2)<br>• From Sweden:<br>○ 7 mg/100 g (coconut fat) | |

*Figure 7* *(cont'd)*



**TABLE 1** (Continued)

Phytosterols concentrations in different food matrices

| Food | Processing conditions | Phytosterol | Concentration | Reference |
|---|---|---|---|---|
| | | Campestanol | • From the Netherlands:<br>◦ 2.7 mg/100 g (baking fat)<br>◦ 4.6 mg/100 g (cooking fat 1)<br>◦ 0.0 mg/100 g (cooking fat 2)<br>◦ 0.0 mg/100 g (frying fat 1)<br>◦ 0.0 mg/100 g (frying fat 2)<br>• From Sweden:<br>◦ 0.0 mg/100 g (coconut fat) | |
| | | Stigmasterol | • From the Netherlands:<br>◦ 56 mg/100 g (baking fat)<br>◦ 29 mg/100 g (cooking fat 1)<br>◦ 22 mg/100 g (cooking fat 2)<br>◦ 25 mg/100 g (frying fat 1)<br>◦ 36 mg/100 g (frying fat 2)<br>• From Sweden:<br>◦ 13 mg/100 g (coconut fat) | |
| | | Sitosterol | • From the Netherlands:<br>◦ 175 mg/100 g (baking fat)<br>◦ 207 mg/100 g (cooking fat 1)<br>◦ 91 mg/100 g (cooking fat 2)<br>◦ 243 mg/100 g (frying fat 1)<br>◦ 127 mg/100 g (frying fat 2)<br>• From Sweden:<br>◦ 36 mg/100 g (coconut fat) | |
| | | Sitostanol | • From the Netherlands:<br>◦ 4.3 mg/100 g (baking fat)<br>◦ 2.9 mg/100 g (cooking fat 1)<br>◦ 0.0 mg/100 g (cooking fat 2)<br>◦ 1.8 mg/100 g (frying fat 1)<br>◦ 2.5 mg/100 g (frying fat 2)<br>• From Sweden:<br>◦ 0.0 mg/100 g (coconut fat) | |
| | | Brassicasterol | • From Netherlands:<br>◦ 5.5 mg/100 g (baking fat)<br>◦ 0.0 mg/100 g (cooking fat 1)<br>◦ 0.0 mg/100 g (cooking fat 2)<br>◦ 5 mg/100 g (frying fat 1)<br>◦ 1.4 mg/100 g (frying fat 2)<br>• From Sweden:<br>◦ 0.0 mg/100 g (coconut fat) | |
| | | Δ-5-Avenasterol | • From the Netherlands:<br>◦ 10.3 mg/100 g (baking fat)<br>◦ 5.1 mg/100 g (cooking fat 1)<br>◦ 6.0 mg/100 g (cooking fat 2)<br>◦ 6.7 mg/100 g (frying fat 1)<br>◦ 7.5 mg/100 g (frying fat 2)<br>• From Sweden:<br>◦ 17.1 mg/100 g (coconut fat) | |
| Wafer biscuit | N/A | Brassicasterol | 0.39–1.67 mg/kg | Hu et al. (2018) |
| | | Campesterol | 13.89–26.36 mg/kg | |
| | | Stigmasterol | 5.20–14.12 mg/kg | |
| | | Sitosterol | 52.50–95.32 mg/kg | |
| Soda biscuit | | Brassicasterol | 0.22–1.62 mg/kg | |
| | | Campesterol | 26.83–36.11 mg/kg | |
| | | Stigmasterol | 6.40–10.83 mg/kg | |
| | | Sitosterol | 27.96–67.87 mg/kg | |

*Figure 7* *(cont'd)*



**TABLE 1** (Continued)

Phytosterols concentrations in different food matrices

| Food | Processing conditions | Phytosterol | Concentration | Reference |
|---|---|---|---|---|
| Cookies | | Brassicasterol | 0.41–1.01 mg/kg | |
| | | Campesterol | 9.27–37.74 mg/kg | |
| | | Stigmasterol | 0.42–13.77 mg/kg | |
| | | Sitosterol | 45.08–104.81 mg/kg | |
| Biscuit | | Brassicasterol | 0.30–30.33 mg/kg | |
| Linseed | N/A | β-sitosterol | 57.4 ± 2.4 mg/100 g | Ryan, Galvin, O'Connor, Maguire, and O'Brien (2007) |
| | | Campesterol | 19.0 ± 0.7 mg/100 g | |
| | | Stigmasterol | 21.8 ± 0.8 mg/100 g | |
| Mustard | | β-sitosterol | 74.4 ± 3.4 mg/100 g | |
| | | Campesterol | 26.5 ± 1.3 mg/100 g | |
| | | Stigmasterol | 2.5 ± 0.3 mg/100 g | |
| Poppy | | β-sitosterol | 58.3 ± 1.0 mg/100 g | |
| | | Campesterol | 9.8 ± 0.4 mg/100 g | |
| | | Stigmasterol | 5.7 ± 0.6 mg/100 g | |
| Pumpkin | | β-sitosterol | 24.9 ± 1.4 mg/100 g | |
| | | Campesterol | ND | |
| | | Stigmasterol | 8.4 ± 0.3 mg/100 g | |
| Sesame | | β-sitosterol | 139.0 ± 7.4 mg/100 g | |
| | | Campesterol | 22.3 ± 1.3 mg/100 g | |
| | | Stigmasterol | 41.5 ± 2.1 mg/100 g | |
| Barley | | β-sitosterol | 38.1 ± 1.0 mg/100 g | |
| | | Campesterol | 12.0 ± 1.0 mg/100 g | |
| | | Stigmasterol | 0.3 ± 0.1 mg/100 g | |
| Buckwhe | | β-sitosterol | 94.5 ± 4.1 mg/100 g | |
| At | | Campesterol | 10.4 ± 0.4 mg/100 g | |
| | | Stigmasterol | 1.6 ± 0.2 mg/100 g | |
| Maize | | β-sitosterol | 34.1 ± 1.1 mg/100 g | |
| | | Campesterol | 9.1 ± 0.5 mg/100 g | |
| | | Stigmasterol | 0.4 ± 0.0 mg/100 g | |
| Millet | | β-sitosterol | 48.3 ± 5.5 mg/100 g | |
| | | Campesterol | 8.7 ± 2.4 mg/100 g | |
| | | Stigmasterol | 0.8 ± 0.3 mg/100 g | |
| Quinoa | | β-sitosterol | 63.7 ± 4.0 mg/100 g | |
| | | Campesterol | 15.6 ± 8.7 mg/100 g | |
| | | Stigmasterol | 3.2 ± 0.1 mg/100 g | |
| Rye | | β-sitosterol | 58.4 ± 5.6 mg/100 g | |
| | | Campesterol | 16.8 ± 1.7 mg/100 g | |
| | | Stigmasterol | 0.7 ± 0.1 mg/100 g | |
| Spelt | | β-sitosterol | 53.3 ± 2.7 mg/100 g | |
| | | Campesterol | 15.1 ± 3.4 mg/100 g | |
| | | Stigmasterol | 0.4 ± 0.0 mg/100 g | |
| Butter | | β-sitosterol | 85.1 ± 7.3 mg/100 g | |
| Beans | | Campesterol | 15.2 ± 2.9 mg/100 g | |
| | | Stigmasterol | 86.2 ± 5.7 mg/100 g | |

*Figure 7* *(cont'd)*



**TABLE 1** (Continued)

Phytosterols concentrations in different food matrices

| Food | Processing conditions | Phytosterol | Concentration | Reference |
|---|---|---|---|---|
| Chick peas | | β-sitosterol | 159.8 ± 7.1 mg/100 g | |
| | | Campesterol | 21.4 ± 0.7 mg/100 g | |
| | | Stigmasterol | 23.4 ± 0.7 mg/100 g | |
| Kidney beans | | β-sitosterol | 86.5 ± 2.6 mg/100 g | |
| | | Campesterol | 6.5 ± 0.8 mg/100 g | |
| | | Stigmasterol | 41.4 ± 1.6 mg/100 g | |
| Lentils | | β-sitosterol | 123.4 ± 4.1 mg/100 g | |
| | | Campesterol | 15.0 ± 0.4 mg/100 g | |
| | | Stigmasterol | 20.0 ± 0.6 mg/100 g | |
| Peas | | β-sitosterol | 191.4 ± 0.4 mg/100 g | |
| | | Campesterol | 25.0 ± 6.9 mg/100 g | |
| | | Stigmasterol | 26.0 ± 0.6 mg/100 g | |
| Wheat (germ) | N/A | β-sitosterol | 2,374 mg/kg fresh weight | Plumb et al. (2011) |
| | | Campesterol | 829 mg/kg fresh weight | |
| | | Stigmasterol | 29 mg/kg fresh weight | |
| | | Δ-5-Avenasterol | 148 mg/kg fresh weight | |
| | | Δ-7-Avenasterol | 90 mg/kg fresh weight | |
| | | Brassicasterol | 24 mg/kg fresh weight | |
| | | Campestanol | 62 mg/kg fresh weight | |
| | | Sitostanol | 84 mg/kg fresh weight | |
| Rape | | β-sitosterol | 1959 mg/kg fresh weight | |
| | | Campesterol | 602 mg/kg fresh weight | |
| | | Stigmasterol | 10 mg/kg fresh weight | |
| | | Δ-5-Avenasterol | 75 mg/kg fresh weight | |
| | | Δ-7-Avenasterol | ND | |
| | | Brassicasterol | 424 mg/kg fresh weight | |
| | | Campestanol | ND | |
| | | Sitostanol | ND | |
| Sunflower | | β-sitosterol | 1,691 mg/kg fresh weight | |
| | | Campesterol | 259 mg/kg fresh weight | |
| | | Stigmasterol | 198 mg/kg fresh weight | |
| | | Δ-5-Avenasterol | 56 mg/kg fresh weight | |
| | | Δ-7-Avenasterol | ND | |
| | | Brassicasterol | ND | |
| | | Campestanol | 41 mg/kg fresh weight | |
| | | Sitostanol | 23 mg/kg fresh weight | |
| Wheat | | β-sitosterol | 858 mg/kg fresh weight | |
| | | Campesterol | 292 mg/kg fresh weight | |
| | | Stigmasterol | 53 mg/kg fresh weight | |
| | | Δ-5-Avenasterol | 29 mg/kg fresh weight | |
| | | Δ-7-Avenasterol | 22 mg/kg fresh weight | |
| | | Brassicasterol | ND | |
| | | Campestanol | 241 mg/kg fresh weight | |
| | | Sitostanol | 288 mg/kg fresh weight | |

*Figure 7* *(cont'd)*



**TABLE 1** (Continued)

Phytosterols concentrations in different food matrices

| Food | Processing conditions | Phytosterol | Concentration | Reference |
|---|---|---|---|---|
| Rye (seed) | | β-sitosterol | 435 mg/kg fresh weight | |
| | | Campesterol | 152 mg/kg fresh weight | |
| | | Stigmasterol | 27 mg/kg fresh weight | |
| | | Δ-5-Avenasterol | ND | |
| | | Δ-7-Avenasterol | 12 mg/kg fresh weight | |
| | | Brassicasterol | 5 mg/kg fresh weight | |
| | | Campestanol | 73 mg/kg fresh weight | |
| | | Sitostanol | 93 mg/kg fresh weight | |
| Wheat (kernel) | | β-sitosterol | 346 mg/kg fresh weight | |
| | | Campesterol | 105 mg/kg fresh weight | |
| | | Stigmasterol | 22 mg/kg fresh weight | |
| | | Δ-5-Avenasterol | 11 mg/kg fresh weight | |
| | | Δ-7-Avenasterol | 6 mg/kg fresh weight | |
| | | Brassicasterol | 25 mg/kg fresh weight | |
| | | Campestanol | 37 mg/kg fresh weight | |
| | | Sitostanol | 56 mg/kg fresh weight | |
| Maize | | β-sitosterol | 313 mg/kg fresh weight | |
| | | Campesterol | 92 mg/kg fresh weight | |
| | | Stigmasterol | 33 mg/kg fresh weight | |
| | | Δ-5-Avenasterol | 23 mg/kg fresh weight | |
| | | Δ-7-Avenasterol | ND | |
| | | Brassicasterol | 5 mg/kg fresh weight | |
| | | Campestanol | 38 mg/kg fresh weight | |
| | | Sitostanol | 100 mg/kg fresh weight | |
| Local Royal Jelly | N/A | Sitosterol | 0.106 mg/mg | Ferioli, Armaforte, and Caboni (2014) |
| | | Δ-5-Avenasterol | 0.123 mg/mg | |
| Commercial Royal Jelly | | Sitosterol | 0.120 mg/mg | |
| | | Δ-5-Avenasterol | 0.214 mg/mg | |
| Soybean flour | N/A | Campestanol | 60.12 ± 0.25 mg/100 g fat | Riciputi et al. (2016) |
| | | Stigmasterol | 50.82 ± 0.85 mg/100 g fat | |
| | | β-sitosterol | 172.23 ± 0.35 mg/100 g fat | |
| | | Δ-7-Avenasterol | 15.11 ± 0.14 mg/100 g fat | |
| Tofu | • Control<br>• Fermented | Campestanol | • 60.42 ± 0.17 mg/100 g fat (control)<br>• 59.53 ± 0.45 mg/100 g fat (fermented) | |
| | | Stigmasterol | • 51.11 ± 0.45 mg/100 g fat (control)<br>• 49.55 ± 0.53 mg/100 g fat (fermented) | |
| | | β-sitosterol | • 156.22 ± 0.45 mg/100 g fat (control)<br>• 168.23 ± 0.19 mg/100 g fat (fermented) | |
| | | Δ-7-Avenasterol | • 15.46 ± 0.19 mg/100 g fat (control)<br>• 17.08 ± 0.06 mg/100 g fat (fermented) | |
| Pomegranate cultivars | Akko | Total Phytosterols | 8.73 ± 0.04 mg/g of oil | Verardo et al. (2014) |
| | Radisa | | 10.45 ± 0.55 mg/g of oil | |
| | Hershkovitz | | 8.98 ± 0.27 mg/g of oil | |
| | Valenciana | | 9.47 ± 0.09 mg/g of oil | |
| | Ravenna | | 9.36 ± 0.09 mg/g of oil | |

*Figure 7* *(cont'd)*



**TABLE 1** (Continued)

Phytosterols concentrations in different food matrices

| Food | Processing conditions | Phytosterol | Concentration | Reference |
|---|---|---|---|---|
| | Veneti | | 10.23 ± 0.08 mg/g of oil | |
| | Hijaz | | 12.15 ± 0.16 mg/g of oil | |
| | Shiraz | | 7.86 ± 0.14 mg/g of oil | |
| | Dente di Cavallo | | 13.22 ± 0.03 mg/g of oil | |
| | Mollar(1) | | 10.63 ± 0.17 mg/g of oil | |
| | Mollar(2) | | 7.46 ± 0.07 mg/g of oil | |
| | Wonderful1 | | 16.42 ± 0.42 mg/g of oil | |
| | Wonderful | | 12.22 ± 0.24 mg/g of oil | |
| | G(1) | | 15.21 ± 0.77 mg/g of oil | |
| | G(2) | | 10.53 ± 0.00 mg/g of oil | |
| | Ecotipo(1) | | 12.73 ± 0.43 mg/g of oil | |
| | Ecotipo(2) | | 12 ± 0.42 mg/g of oil | |
| Buckwheat flour | N/A | Total Phytosterols | 99 mg/100 g edible portion | Normén et al. (2002) |
| Cornflour | | | 52 mg/100 g edible portion | |
| Couscous | • Raw<br>• Cooked | | • 58 mg/100 g edible portion (raw)<br>• 24 mg/100 g edible portion (cooked) | |
| Flour blend | • Wheat, rye<br>• Wheat, rye, barley, oat<br>• Coarse wheat, rye | | • 47 mg/100 g edible portion (wheat, rye)<br>• 47 mg/100 g edible portion (wheat, rye, barley, oat)<br>• 78 mg/100 g edible portion (coarse wheat, rye) | |
| Millet | Cooked | | 55 mg/100 g edible portion | |
| Rice | • Flour<br>• Brown, cooked<br>• Jasmine, cooked<br>• Parboiled, cooked | | • 23 mg/100 g edible portion (flour)<br>• 29 mg/100 g edible portion (brown, cooked)<br>• 21 mg/100 g edible portion (jasmine, cooked)<br>• 39 mg/100 g edible portion (parboiled, cooked) | |
| Rye | • Crushed grains<br>• Flour | | • 69 mg/100 g edible portion (crushed grains)<br>• 86 mg/100 g edible portion (flour) | |
| Semolina | Cooked | | 17 mg/100 g edible portion | |
| Whole wheat flour | N/A | | 70 mg/100 g edible portion | |
| Wheat | • Crushed grains<br>• Flour<br>• Germs | | • 69 mg/100 g edible portion (crushed grains)<br>• 28 mg/100 g edible portion (flour)<br>• 344 mg/100 g edible portion (germs) | |
| Bambix (mixed cereals for porridge) | N/A | Total Phytosterols | 73 mg/100 g edible portion | |
| Buckwheat flakes | | | 94 mg/100 g edible portion | |
| Branflakes | | | 65 mg/100 g edible portion | |
| Corn flakes | • Normal<br>• Frosties | | • 26 mg/100 g edible portion (normal)<br>• 17 mg/100 g edible portion (frosties) | |
| Corn starch | N/A | | 4.1 mg/100 g edible portion | |
| Gruel meal corn | • Raw<br>• Cooked | | • 64 mg/100 g edible portion (raw)<br>• 16 mg/100 g edible portion (cooked) | |
| Gruel meal wheat | • Raw<br>• Cooked | | • 43 mg/100 g edible portion (raw)<br>• 13 mg/100 g edible portion (cooked) | |

*Figure 7* *(cont'd)*



**TABLE 1** (Continued)

Phytosterols concentrations in different food matrices

| Food | Processing conditions | Phytosterol | Concentration | Reference |
|---|---|---|---|---|
| Gruel meal wholemeal | • Raw<br>• Cooked | | • 56 mg/100 g edible portion (raw)<br>• 14 mg/100 g edible portion (cooked) | |
| Musli | • Without sugar added<br>• Fruit, with low sugar content<br>• With high sugar content | | • 35 mg/100 g edible portion (without sugar added)<br>• 49 mg/100 g edible portion (fruit, with low sugar content)<br>• 105 mg/100 g edible portion (with high sugar content) | |
| Oat | • Bran<br>• Crunches | | • 46 mg/100 g edible portion (bran)<br>• 39 mg/100 g edible portion (crunches) | |
| Oat must | • Raw<br>• Cooked | | • 38 mg/100 g edible portion (raw)<br>• 5.5 mg/100 g edible portion (cooked) | |
| Pasta | Cooked | | 36 mg/100 g edible portion | |
| Rice | • Krispies<br>• Puffed, roasted | | • 24 mg/100 g edible portion (krispies)<br>• 20 mg/100 g edible portion (puffed, roasted) | |
| Rolled oats | • Raw<br>• Cooked<br>• With added fiber, raw<br>• With added fiber, cooked | | • 39 mg/100 g edible portion (raw)<br>• 5.0 mg/100 g edible portion (cooked)<br>• 54 mg/100 g edible portion (with added fiber, raw)<br>• 14 mg/100 g edible portion (with added fiber, cooked) | |
| Special K | N/A | | 40 mg/100 g edible portion | |
| Weetabix | | | 94 mg/100 g edible portion | |
| Wheat | • Bran<br>• Bran, coarse<br>• Flakes | | • 200 mg/100 g edible portion (bran)<br>• 197 mg/100 g edible portion (bran, coarse)<br>• 68 mg/100 g edible portion (flakes) | |
| Bread crumbs | N/A | | 56 mg/100 g edible | |
| Dutch rusks | | | 53 mg/100 g edible | |
| Rye bread | • Light<br>• Dark | | • 51 mg/100 g edible (light)<br>• 51 mg/100 g edible (dark) | |
| Swedish hard bread | N/A | | 89 mg/100 g edible | |
| Wheat bread | • Traditional<br>• Made with water<br>• Made with milk | | • 54 mg/100 g edible (Riciputi et al.)<br>• 50 mg/100 g edible (made with water)<br>• 29 mg/100 g edible (made with milk) | |
| Wholemeal bread | N/A | | 86 mg/100 g edible | |
| Chocolate biscuit | | Campesterol | 8.3 mg / 100 g | |
| | | Campestanol | 0.6 mg / 100 g | |
| | | Stigmasterol | 14.5 mg /100 g | |
| | | Sitosterol | 43.5 mg /100 g | |
| | | Sitostanol | 1.0 mg / 100 g | |
| | | Brassicasterol | 0.4 mg / 100 g | |
| | | Δ-5-Avenasterol | 1.8 mg / 100 g | |
| Chocolate | • Dark<br>• Milk | Campesterol | • 12.3 mg / 100 g (dark)<br>• 10 mg / 100 g (milk) | |
| | | Campestanol | • 0.0 mg /100 g (dark)<br>• 0.5 mg /100 g (milk) | |
| | | Stigmasterol | • 31.4 mg / 100 g (dark)<br>• 23 mg / 100 g (milk) | |
| | | Sitosterol | • 77.6 mg / 100 g (dark)<br>• 56 mg / 100 g (milk) | |

*Figure 7* *(cont'd)*



**TABLE 1** (Continued)

Phytosterols concentrations in different food matrices

| Food | Processing conditions | Phytosterol | Concentration | Reference |
|---|---|---|---|---|
| | | Sitostanol | • 1.0 mg / 100 g (dark)<br>• 0.9 mg / 100 g (milk) | |
| | | Brassicasterol | • 0.9 mg / 100 g (dark)<br>• 0.9 mg / 100 g (milk) | |
| | | Δ-5-Avenasterol | • 3.1 mg / 100 g (dark)<br>• 2.5 mg / 100 g (milk) | |
| Crisps | | Campesterol | 15 mg /100 g | |
| | | Campestanol | 0.7 mg /100 g | |
| | | Stigmasterol | 14 mg /100 g | |
| | | Sitosterol | 45 mg /100 g | |
| | | Sitostanol | 1.1 mg /100 g | |
| | | Brassicasterol | 2.4 mg /100 g | |
| | | Δ-5-Avenasterol | 8.9 mg /100 g | |
| Flapjacks | | Campesterol | 5.2 mg /100 g | |
| | | Campestanol | 0.0 mg /100 g | |
| | | Stigmasterol | 3.2 mg /100 g | |
| | | Sitosterol | 32.8 mg /100 g | |
| | | Sitostanol | 0.6 mg /100 g | |
| | | Brassicasterol | 0.0 mg /100 g | |
| | | Δ-5-Avenasterol | 5.6 mg /100 g | |
| Ice cream | • Non-dairy<br>• Soy based, non-diary | Campesterol | • 12.3 mg /100 g (non-dairy)<br>• 10 mg / 100 g (soy based, non-dairy) | |
| | | Campestanol | • 0.0 mg / 100 g (non-dairy)<br>• 0.5 mg / 100 g (soy based, non-dairy) | |
| | | Stigmasterol | • 31.4 mg /100 g (non-dairy)<br>• 23 mg / 100 g (soy based, non-dairy) | |
| | | Sitosterol | • 77.6 mg /100 g (non-dairy)<br>• 56 mg / 100 g (soy based, non-dairy) | |
| | | Sitostanol | • 1.0 mg / 100 g (non-dairy)<br>• 0.9 mg / 100 g (soy based, non-dairy) | |
| | | Brassicasterol | • 0.9 mg / 100 g (non-dairy)<br>• 0.9 mg / 100 g (soy based, non-dairy) | |
| | | Δ-5-Avenasterol | • 3.1 mg / 100 g (non-dairy)<br>• 2.5 mg / 100 g (soy based, non-dairy) | |
| Mayonaise | Rapeseed oil based | Campesterol | 181 mg / 100 g | |
| | | Campestanol | 0.0 mg /100 g | |
| | | Stigmasterol | 24 mg /100 g | |
| | | Sitosterol | 251 mg / 100 g | |
| | | Sitostanol | 2.1 mg /100 g | |
| | | Brassicasterol | 5.1 mg /100 g | |
| | | Δ-5-Avenasterol | 20.9 mg /100 g | |
| Peanut butter | • From the Netherlands<br>• From Sweden | Campesterol | • 33 mg / 100 g (from the Netherlands)<br>• 20 mg / 100 g (from Sweden) | |
| | | Campestanol | • 0.0 mg / 100 g (from the Netherlands)<br>• 0.0 mg / 100 g (from Sweden) | |
| | | Stigmasterol | • 26 mg / 100 g (from the Netherlands)<br>• 20 mg / 100 g (from Sweden) | |
| | | Sitosterol | • 121 mg /100 g (from the Netherlands)<br>• 100 mg /100 g (from Sweden) | |

*Figure 7* *(cont'd)*



**TABLE 1** (Continued)

| Phytosterols concentrations in different food matrices | | | | |
|---|---|---|---|---|
| **Food** | **Processing conditions** | **Phytosterol** | **Concentration** | **Reference** |
| | | Sitostanol | • 2.2 mg /100 g (from the Netherlands)<br>• 0.0 mg /100 g (from Sweden) | |
| | | Brassicasterol | • 3.6 mg /100 g (from the Netherlands)<br>• 0.0 mg /100 g (from Sweden) | |
| | | Δ-5-Avenasterol | • 11.7 mg /100 g (from the Netherlands)<br>• 20.2 mg /100 g (from Sweden) | |
| Salad dressing | • From the Netherlands<br>○ Becel dressing<br>○ Fritessaus<br>○ Slasaus | Campesterol | • 16 mg /100 g (Becel dressing)<br>• 139 mg /100 g (Fritessaus)<br>• 29 mg /100 g (Slasaus) | |
| | | Campestanol | • 2.7 mg /100 g (Becel dressing)<br>• 0.0 mg /100 g (Fritessaus)<br>• 0.0 mg /100 g (Slasaus) | |
| | | Stigmasterol | • 15 mg /100 g (Becel dressing)<br>• 2 mg /100 g (Fritessaus)<br>• 13 mg /100 g (Slasaus) | |
| | | Sitosterol | • 108 mg /100 g (Becel dressing)<br>• 176 mg /100 g (Fritessaus)<br>• 53 mg /100 g (Slasaus) | |
| | | Sitostanol | • 0.0 mg /100 g (Becel dressing)<br>• 0.0 mg /100 g (Fritessaus)<br>• 0.0 mg /100 g (Slasaus) | |
| | | Brassicasterol | • 2.3 mg /100 g (Becel dressing)<br>• 31 mg /100 g (Fritessaus)<br>• 7.3 mg /100 g (Slasaus) | |
| | | Δ-5-Avenasterol | • 8.8 mg /100 g (Becel dressing)<br>• 13.9 mg /100 g (Fritessaus)<br>• 3.2 mg /100 g (Slasaus) | |
| *Others* | | | | |
| Recuperation bread | N/A | Sitosterol | 13.09–85.75 mg/kg | Hu et al. (2018) |
| | | Brassicasterol | 0.02–1.75 mg/kg | |
| | | Campesterol | 1.33–25.84 mg/kg | |
| | | Stigmasterol | 0.69–18.17 mg/kg | |
| | | Sitosterol | 1.34–42.27 mg/kg | |
| French fries | • Control<br>• Heating (oven for 15 min @ 225° C) | Brassicasterol | • 41.3 ± 2.9 mg/100 g fat (control)<br>• 36.8 ± 3.3 mg/100 g fat (heated) | Derewiaka and Obiedziński (2012) |
| | | Campesterol | • 121.4 ± 6.0 mg/100 g fat (control)<br>• 108.3 ± 12.7 mg/100 g fat (heated) | |
| | | Stigmasterol | • 73.8 ± 4.1 mg/100 g fat (control)<br>• 65.6 ± 2.6 mg/100 g fat (heated) | |
| | | Sitosterol | • 263.3 ± 18.1 mg/100 g fat (control)<br>• 197.6 ± 14.5 mg/100 g fat (heated) | |
| Chocolate bar | Storage (20 and 30°C for 5 months) | Stigmasterol | 0.01 ± 0.00 g/bar | Borges Botelho et al. (2014) |
| | | β-sitosterol | 0.02 ± 0.00 g/bar | |
| Chocolate bar + palm oil | | Brassicasterol | 0.08 ± 0.00 g/bar | |
| | | Stigmasterol | 0.37 ± 0.00 g/bar | |
| | | β-sitosterol | 1.08 ± 0.01 g/bar | |
| | | Campesterol | 0.59 ± 0.00 g/bar | |

*Figure 7* (cont'd)



**TABLE 1** (Continued)

Phytosterols concentrations in different food matrices

| Food | Processing conditions | Phytosterol | Concentration | Reference |
|---|---|---|---|---|
| Chocolate bar + palm oil + ascorbic Acid and α-tocopherol) | | Brassicasterol | 0.09 ± 0.00 g/bar | |
| | | Stigmasterol | 0.42 ± 0.01 g/bar | |
| | | β-sitosterol | 1.21 ± 0.02 g/bar | |
| | | Campesterol | 0.67 ± 0.01 g/bar | |
| Sponge cake, banana bread, muffins and cookies | N/A | Total Phytosterols | 15.0–55.1 mg sterols/100 g of ingredient | Menéndez-Carreño et al. (2016) |
| Brownie | | | 0.611 ± 0.02 g/100 g | Srigley and Haile (2015) |
| Whole wheat pita | | | 1.16 ± 0.06 g/100 g | |
| Oatmeal square-1 | | | 1.50 ± 0.07 g/100 g | |
| Oatmeal square-2 | | | 1.52 ± 0.07 g/100 g | |
| Oatmeal cookie | | | 2.70 ± 0.08 g/100 g | |
| Apple cake | N/A | | 27 | Normén et al. (2002) |
| Biscuits, salted | | | 52 | |
| Cake | • Swedish<br>• Cream<br>• Cream layered | | • 112 mg/100 g edible portion (Swedish)<br>• 28 mg/100 g edible portion (cream)<br>• 29 mg/100 g edible portion (cream layered) | |
| Crackers | Sweet | | 96 mg/100 g edible portion<br>49 mg/100 g edible portion | |
| Digestive crackers | N/A | | 55 mg/100 g edible portion | |
| Fruit pie | | | 30 mg/100 g edible portion | |
| Gingerbread biscuits | | | 71 mg/100 g edible portion | |
| Marzipan pastry | | | 62 mg/100 g edible portion | |
| Maryland cookies | | | 68 mg/100 g edible portion | |
| Pastry | • Danish<br>• Puffed, salt | | • 61 mg/100 g edible portion (Danish)<br>• 36 mg/100 g edible portion (puffed, salt) | |
| Sweet biscuits, pool Dutch sample | N/A | | 55 mg/100 g edible portion | |
| Wafer chocolate | | | 44 mg/100 g edible portion | |
| Wheat cinnamon buns | | | 48 mg/100 g edible portion | |
| *Bakery products* | | | | |
| Biscuit | N/A | Campesterol | 12.04–102.92 mg/kg | Hu et al. (2018) |
| | | Stigmasterol | 0.30–12.49 mg/kg | |
| | | Sitosterol | 48.81–92.84 mg/kg | |
| Sandwich biscuit | | Brassicasterol | 0.27–0.93 mg/kg | |
| | | Campesterol | 16.55–38.57 mg/kg | |
| | | Stigmasterol | 5.31–15.39 mg/kg | |
| | | Sitosterol | 41.60–81.70 mg/kg | |
| Egg roll | | Brassicasterol | 0.39–0.91 mg/kg | |
| | | Campesterol | 14.63–37.45 mg/kg | |
| | | Stigmasterol | 3.24–19.12 mg/kg | |
| | | Sitosterol | 50.72–81.88 mg/kg | |
| Egg pie | | Brassicasterol | 0.15–0.96 mg/kg | |
| | | Campesterol | 5.69–33.03 mg/kg | |
| | | Stigmasterol | 1.52–27.28 mg/kg | |
| | | Sitosterol | 15.48–67.92 mg/kg | |

*Figure 7* *(cont'd)*



**TABLE 1** (Continued)

Phytosterols concentrations in different food matrices

| Food | Processing conditions | Phytosterol | Concentration | Reference |
|---|---|---|---|---|
| Caramel treats | | Brassicasterol | 0.27–1.17 mg/kg | |
| | | Campesterol | 17.29–30.29 mg/kg | |
| | | Stigmasterol | 4.01–9.16 mg/kg | |
| | | Sitosterol | 68.45–113.17 mg/kg | |
| Cake | | Brassicasterol | 0.14–0.79 mg/kg | |
| | | Campesterol | 10.72–35.86 mg/kg | |
| | | Stigmasterol | 5.59–35.93 mg/kg | |
| | | Sitosterol | 10.68–47.88 mg/kg of | |
| Egg tart | | Brassicasterol | 0.18–0.41 mg/kg of | |
| | | Campesterol | 4.91–11.12 mg/kg of | |
| | | Stigmasterol | 1.90–4.83 mg/kg of | |
| | | Sitosterol | 16.93–36.18 mg/kg | |
| Staple bread | | Brassicasterol | 0.05–0.40 mg/kg | |
| | | Campesterol | 2.75–24.83 mg/kg | |
| | | Stigmasterol | 1.52–10.97 mg/kg | |
| | | Sitosterol | 4.53–60.26 mg/kg | |
| Fancy bread | | Brassicasterol | 0.19–0.83 mg/kg | |
| | | Campesterol | 9.44–35.32 mg/kg | |
| | | Stigmasterol | 3.39–21.78 mg/kg | |

*Note*: Results are mean values ± *SD*.
Abbreviations: N/A = not applicable; ND = not detected.

culture. In Northern Europe, up to 40% of dietary PS derive from cereals and cereal-based foods. However, in countries having greater availability of fresh fruits the main contributor to PS in the diet are fruits and vegetables, contributing as much as 35% of dietary PS, as a study performed in Uruguay showed (Lea, Hepburn, Wolfreys, & Baldrick, 2004; Piironen et al., 2000). Databases of almost every type of food have been developed over the years, and even country-specific food items have been analyzed such as the ones reported by (Normén et al., 2007) which refers to the amount of PS found in spreads, oils, seeds, and various other fatty foods typically consumed in Sweden and the Neatherlands. PS content in foods has been summarized in Table 1. Animal origin food items are included because of the addition of vegetable oils or ingredients as part of different food processing techniques. However, the previously mentioned variability of PS concentration in animal origin food items, based on the plant's origin of the additive is strongly noticeable between food items. Some differences are due to the supplementation of plant-based oils or even because of the various diets of cows, pigs, chickens, and other animals typically consumed by humans, which contain plant products.

## 3 | HEALTH BENEFITS OF PHYTOSTEROLS

The chemical similarity of PS to cholesterol has played an important role in the study and analysis of the physiological aspects of PS. Most of its chemical properties have been studied based on the information known for cholesterol (Maldonado-Pereira, Schweiss, Barnaba, & Medina-Meza, 2018). Absorption of PS in humans is low compared to cholesterol, ranging from 2 to 5% of total intake, versus 60%, respectively, (Alemany et al., 2014). Mellies et al. (1976) performed a study on infants and children to see the effects of dietary phytosterols. They found that infants fed with infant formulas enriched with phytosterols had blood plasma levels of campesterol and β-sitosterol that were three to five times higher than infants fed with breast or cow's milk. At the time when this initial quantitative assessment was performed, little was known about the potential biological implications of phytosterols in infants (Mellies et al., 1976; Ostlund Jr, 2002). Due to their structural similarity to cholesterol, PS were first and foremost studied for their cholesterol absorption inhibition properties. They are well known for their ability to reduce cholesterol absorption (Nestel, Cehun, Pomeroy, Abbey, & Weldon, 2001; Ostlund Jr, 2002; Ostlund Jr, Racette, Okeke, & Stenson, 2002; Ostlund Jr, Racette, & Stenson, 2003), which is reflected in a reduced cholesterol plasma concentration (Law, 2000; Nguyen, 1999; Piironen et al., 2000; Pinedo et al., 2007); lipoprotein oxidation reduction anti-inflammatory properties (Gabay, Lamacchia, & Palmer, 2010), anti-cancer properties, and apoptosis induction, positive regulation on testosterone metabolism (Awad, Tagle Hernandez, Fink, & Mendel, 1997), cancer cell proliferation reduction by angiogenesis inhibition (Lea et al., 2004; Shahzad

*Figure 7* *(cont'd)*



et al., 2017), and tumor growth reduction (Danesi et al., 2011; Llaverias et al, 2013). The National Cholesterol Education Program recommends adding 2 g/day of phytosterols to the diet to reduce LDL cholesterol concentrations and coronary heart disease risk (NCEP, 2001). In 2000, the FDA issued an interim rule allowing the claim that plant stanyl and steryl esters-containing foods reduce the risk of coronary heart disease, because their demonstrated cholesterol-lowering effect (Golley & Hendrie, 2014; Moreau et al., 2002). Moreover, PS success in health has been proved by the development of different patents and commercial PS products currently being marketed worldwide (García-Llatas & Rodríguez-Estrada, 2011; González-Larena et al., 2011).

## 4 | PHYTOSTEROLS OXIDATION

PS are particularly susceptible to oxidation due to their surface activation property, by exposure to UV light, high temperatures, and/or oxygen sources. For example, oil refining processes diminish PS content by 2–5%, increasing the oxidation as consequence. The presence of heat, light, metal contaminants catalyze radical-mediated oxidation at the double bond, starting an autocatalytic oxidative chain reaction. Similarly to general lipid oxidation processes, PS oxidation evolves through three main steps: the *initiation* corresponding to the generation of highly-reactive radical species, the *propagation* of radical species via autocatalysis, and the *termination* of the reactions with consequent formation of thermodynamically stable compounds (Johnson & Decker, 2015). In foods, free radicals can be generated by photosensitization mediated by either chlorophylls (vegetable matrices; Medina-Meza, Barnaba, & Barbosa-Cánovas, 2014) or heme (animal matrices), which leads to the formation of singlet oxygen, or by reacting with metals (Boatright & Crum, 2016). Oxidation can also occur by pre-existing reactive oxygen species (ROS) and oxidative enzymes such as cytochrome P450, superoxides, and peroxidases naturally present in vegetable matrices (Ryan, McCarthy, Maguire, & O'Brien, 2009). The oxidation of PS in food follows similar chemical pathways to cholesterol oxidation, and the products of PS oxidation are known as phytosterol oxidation products (POPs; Medina-Meza & Barnaba, 2013). PS major oxidation pathways are shown in Figure 2. Primary products of oxidation are the allylic 7-hydroperoxides, which are further oxidized to the corresponding 7α- and 7β-hydroxy by epimerization. Hydroxyls can be further oxidized to the chemically stable 7-keto compounds, as well as the highly reactive 5,6α- and 5,6β-epoxy compounds. The epoxides generally are hydrolyzed to form the 3,5,6β-triols (Barriuso, Otaegui-Arrazola, Menéndez-Carreño, Astiasarán, & Ansorena, 2012; Cercaci, Rodriguez-Estrada, Lercker, & Decker, 2007; González-Larena et al., 2011; Lin et al., 2017; Lin et al., 2018; O'Callaghan, McCarthy, & O'Brien, 2014). Main factors promoting POPs formation in foods are: (a) processing temperature and time, (b) storage temperature: affects only when temperature is over 34°C, (c) sterols structure, (d) esterification, (e) degree of saturation, and (f) food matrix (Danesi et al., 2011). The concentration of POPs in various food products is shown in Table 2. POPs formation increases proportionally to temperature and time; POPs formation is also affected by water content and oil drop size in the food matrix (Cercaci et al., 2007; McClements, Decker, & Weiss, 2007). Moreover, when producing rapeseed oil industrially, it was found that the refined oil had over double the POP content (Rudzińska, Uchman, & Wąsowicz, 2005).

## 5 | POPS BIOLOGICAL TOXICITY

Few studies have given some insight about POPs and their biological activity both in humans and animal models. Studies performed in animals have demonstrated that POPs can be absorbed at a higher rate compared to phytosterols (Grandgirard, Sergiel, Nour, Demaison-Meloche, & Gihiès, 1999; Meynier, Andre, Lherminier, Grandgirard, & Demaison, 2005). POPs distribution and accumulation in different animal tissues (aorta, heart, kidneys, liver) revealed the highest concentration of hydroxyl derivatives in the liver (Bang, Arakawa, Takada, Sato, & Imaizumi, 2008; Liang et al., 2011; Tomoyori et al., 2004), but triols were also found in the liver, kidney, and the heart lipid fraction. For example, lymphatic recoveries for campesterol oxides (16%) and sitosterol oxides (9%) were higher than for campesterol (6%) and β-sitosterol (2%; Grandgirard, Demaison-Meloche, et al., 2004; Grandgirard, Martine, et al., 2004). These trends are similar to the absorption of non-oxidized phytosterols, implying that increasing the side chain length of either the PS or POPs, decreased their absorption and that the type of oxidation relates to the degree of absorption (Ryan et al., 2009).

POPs may be related to the inflammation processes, dyslipidemia, atherosclerosis, apoptosis, and cell toxicity (Alemany et al., 2014; Hu et al., 2018; Zunin, Calcagno, & Evangelisti, 1998). Adcox, Boyd, Oehrl, Allen, and Fenner (2001) demonstrated that POPs affect protein synthesis and damage the cell membrane, while measuring total protein content and LDL leakage (Adcox et al., 2001). Recently, it has been hypothesized that due to the structural similarity between POPs and cholesterol oxidation products (COPs), POPs could potentially contribute to the onset of metabolic and neurologic diseases by an irreversible accumulation in the central nervous system. A recent study discovered that sitosterol and 7β-hydroxysitosterol can pass through the blood brain barrier (Schött et al., 2015, 2017). Maguire, Konoplyannikov, Ford, Maguire, and O'brien (2003) reported that thermally oxidized derivatives of β-sitosterol have shown similar patterns of toxicity towards a human monocytic cell line (U937) as the cholesterol-derivative 7β-hydroxycholesterol (30 μM), although higher concentrations of the POPs mixture (120 μM) was required. Studies with several cell lines that is, human monocytic (U937), colonic adenocarcinoma (Caco-2) and hepatoma (HepG2) cells, found that 7β-hydroxy, 7-keto and triol derivatives of β-sitosterol were moderately cytotoxic (at 60 and 120 μM) to all three cell lines; the mode of cell death was apoptosis in the U937 cell line and necrosis in the Caco-2 and HepG2 cells (Ryan et al., 2007).

*Figure 7* *(cont'd)*



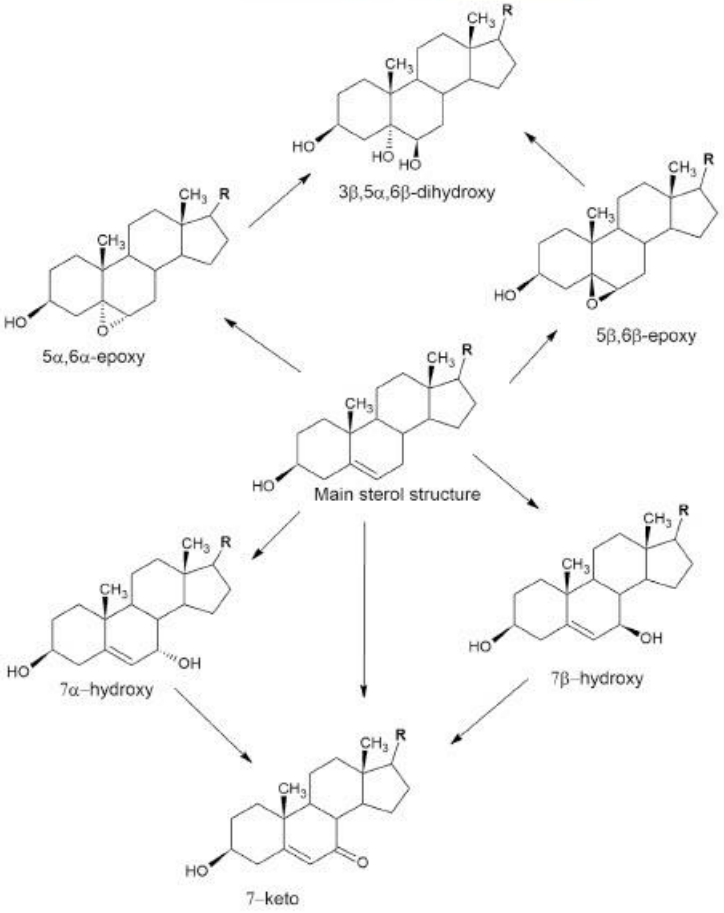


**FIGURE 2** General phytosterol oxidation pathway (R denotes a specific phytosterol side chain)

## 6 | INFANT FORMULATIONS: A CRITICAL NEED

The United States Food and Drug Administration (FDA) defines Infant formula (IF) as "a food which purports to be or is represented for special dietary use solely as a food for infants by reason of its simulation of human milk or its suitability as a complete or partial substitute for human milk" ("Guidance for Industry", 2018). IF provides all nutrients and sustenance for the growth and development of infants when breast feeding is not an option for physiological or medical reasons (Green Corkins & Shurley, 2016; Su et al., 2017; Vandenplas, Zakharova, & Dmitrieva, 2015).

Different health issues, such as allergies and metabolic disorders, that infants could develop during their first months of life, are targeted in the production of numerous types formulas that provide the appropriate nutrition for every child, adding or removing certain components of the infant formula based on the specific need of the infant (Maldonado, Gil, Narbona, & Molina, 1998). In the United States, common types of infant formula are cow's milk-based, soy-based, lactose-reduced or partially hydrolyzed, and specialty. These special formulas are developed for infants who have certain conditions, like protein sensitivity, acid reflux, pre-term, phenylketonuria, and so on. (Rossen, Simon, & Herrick, 2016). Since the infant's nutrition plays a crucial role in development both short and long term (Lemaire, Le Huërou-Luron, & Blat, 2018), this is the main reason why the FDA issued a Code of Federal Regulations (Title 21, Chapter I, Subchapter B, Part 107) where the requirements are outlined for the composition of IF. As it is stated by the FDA, for each 100 cal serving there has to be 300 mg of Linoleic acid and 1.8–4.5 g of protein per serving ("Code of Federal Regulations", 2019). In addition, IF contains around 20% fat, which comes from vegetable oils like sunflower, palm, soy, or coconut oils (5, 8, 7, and 5%, respectively; Desic & Birlouez-Aragon, 2011; Hamdan, Claumarchirant, Garcia-Llatas, Alegría, & Lagarda, 2017; Hamdan, Sanchez-Siles, Garcia-Llatas, & Lagarda, 2018; Rajasekaran & Kalaivani, 2013). PS content in vegetables oils normally added to IF can be seen in Table 3. Sterols content in human milk differ from IF's sterols

*Figure 7* *(cont'd)*



**TABLE 2** Phytosterols oxidation products (POPs) content in different food matrices

| Phytosterols oxidation products (POPs) concentrations in different food matrices | | | | |
|---|---|---|---|---|
| **Food** | **Processing conditions** | **POPs** | **POPs concentration** | **Reference** |
| *Oils* | | | | |
| Sunflower | Heating (16 min @ 210°C) | Sitosterol-POP | 10.3 ± 0.4 mg/100 g fat | Lin et al. (2017) |
| | | Campesterol-POP | 2.1 ± 0.1 mg/100 g fat | |
| Rapeseed | | Sitosterol-POP | 9.2 ± 0.1 mg/100 g fat | |
| | | Campesterol-POP | 7.6 ± 0.1 mg/100 g fat | |
| Soy | • Control<br>• Heating (240 min @ 180°C) | 7α-hydroxysterols | • ND μg/g (control)<br>• 51.69 μg/g (240 min @ 180°C) | Lebovics et al. (1999) |
| | | 7β-hydroxysterols | • ND μg/g sample (control)<br>• 54.39 μg/g (240 min @ 180°C) | |
| | | 7-ketosterols | • ND μg/g (control)<br>• 42.04 μg/g (240 min @ 180°C) | |
| Olive | | 7α-hydroxysterols | • ND μg/g (control)<br>• 36.28 μg/g (240 min @ 180°C) | |
| | | 7β-hydroxysterols | • ND μg/g (control)<br>• 36.43 μg/g (240 min @ 180°C) | |
| | | 7-ketosterols | • ND μg/g (control)<br>• 21.44 μg/g (240 min @ 180°C) | |
| Corn | | 7α-hydroxysterols | • ND μg/g (control)<br>• 80.70 μg/g (240 min @ 180°C) | |
| | | 7β-hydroxysterols | • ND μg/g (control)<br>• 91.00 μg/g (240 min @ 180°C) | |
| | | 7-ketosterols | • ND μg/g (control)<br>• 24.42 μg/g (240 min @ 180°C) | |
| Peanut | | 7α-hydroxysterols | • ND μg/g (control)<br>• 65.50 μg/g (240 min @ 180°C) | |
| | | 7β-hydroxysterols | • ND μg/g (control)<br>• 66.40 μg/g (240 min @ 180°C) | |
| **Phytosterols oxidation products (POPs) concentrations in different food matrices (Borges Botelho et al.)** | | | | |
| **Food** | **Processing conditions** | **POPs** | **POPs concentration** | **Reference** |
| *Oils* | | | | |
| Peanut | • Control<br>• Heating (240 min @ 180°C) | 7-ketosterols | • ND (control)<br>• 33.09 μg/g (240 min @ 180°C) | Lebovics et al. (1999) |
| | • Control<br>• Heating (for 2 hr @ 180 ± 5°C) | 7α-hydroxycampesterol | • 0.3 μg/g oil (control)<br>• 0.3 μg/g oil (2 hr @ 180 ± 5°C) | Johnsson and Dutta (2006) |
| | | 7α-hydroxystigmasterol | • 0.3 μg/g oil (control)<br>• 0.3 μg/g oil (2 hr @ 180 ± 5°C) | |
| | | 7α-hydroxysitosterol | • 0.4 μg/g oil (control)<br>• 0.8 μg/g oil (2 hr @ 180 ± 5°C) | |
| | | 7β-hydroxycampesterol | • 0.2 μg/g oil (control)<br>• ND (240 min @ 180°C) | |
| | | 7β-hydroxystigmasterol | • 0.4 μg/g oil (control)<br>• 0.2 μg/g oil (2 hr @ 180 ± 5°C) | |
| | | 7β-hydroxysitosterol | • 0.6 μg/g oil (control)<br>• 0.9 μg/g oil (2 hr @ 180 ± 5°C) | |

*Figure 7* *(cont'd)*



**TABLE 2** (Continued)

Phytosterols oxidation products (POPs) concentrations in different food matrices (Borges Botelho et al.)

| Food | Processing conditions | POPs | POPs concentration | Reference |
|---|---|---|---|---|
| | | 24-hydroxystigmasterol | • ND (control)<br>• ND (240 min @ 180°C) | |
| | | 24-hydroxycampesterol | • 0.4 μg/g oil (control)<br>• 0.7 μg/g oil (2 hr @ 180 ± 5°C) | |
| | | 24-hydroxysitosterol | • 0.2 μg/g oil (control)<br>• ND (240 min @ 180°C) | |
| | | 5β,6β-epoxycampesterol | • ND (control)<br>• 0.2 μg/g oil (2 hr @ 180 ± 5°C) | |
| | | 5α,6α-epoxycampesterol | • 0.3 μg/g oil (control)<br>• 0.2 μg/g oil (2 hr @ 180 ± 5°C) | |
| | | 5β,6β-epoxystigmasterol | • ND (control)<br>• ND (240 min @ 180°C) | |
| | | 5α,6α-epoxystigmasterol | • ND (control)<br>• ND (240 min @ 180°C) | |
| *Oils* | | | | |
| Peanut | • Control<br>• Heating (for 2 hr @ 180 ± 5°C) | 5β,6β-epoxysitosterol | • ND (control)<br>• 0.2 μg/g oil (2 hr @ 180 ± 5°C) | Johnsson and Dutta (2006) |
| | | 5α,6α-epoxysitosterol | • 0.3 μg/g oil (control)<br>• 0.3 μg/g oil (2 hr @ 180 ± 5°C) | |
| | | Campestanetriol | • 0.8 μg/g oil (control)<br>• ND | |
| | | Stigmastanetriol | • ND (control)<br>• 0.6 μg/g oil (2 hr @ 180 ± 5°C) | |
| | | Sitostanetriol | • 0.9 μg/g oil (control)<br>• ND (240 min @ 180°C) | |
| | | 7-ketocampesterol | • ND (control)<br>• 0.3 μg/g oil (2 hr @ 180 ± 5°C) | |
| | | 7-ketostigmasterol | • 0.7 μg/g oil (control)<br>• 0.5 μg/g oil (2 hr @ 180 ± 5°C) | |
| | | 7-ketositosterol | • 1.3 μg/g oil (control)<br>• 1.3 μg/g oil (2 hr @ 180 ± 5°C) | |
| Rapeseed oil/palm oil blend (RP, GS, KM, and A) | Heating (frying for 15 min @ 200°C) | 7α-hydroxycampesterol | 1.4 μg/g | Dutta and Appelqvist (1997) |
| | | 7β-hydroxycampesterol | 2.6 μg/g | |
| | | 7-ketocampesterol | 3.3 μg/g | |
| | | Epoxycampesterol[a] | 5.4 μg/g | |
| | | Triolcampesterol | 0.5 μg/g | |
| | | 7α-hydroxysitosterol | 2.9 μg/g | |
| | | 7β-hydroxysitosterol | 3.7 μg/g | |
| | | 7-ketositosterol | 4.1 μg/g | |
| | | Epoxysitosterol[a] | 7.6 μg/g | |
| | | Triolsitosterol | 0.5 μg/g | |

*Figure 7* *(cont'd)*



**TABLE 2** (Continued)

Phytosterols oxidation products (POPs) concentrations in different food matrices (Borges Botelho et al.)

| Food | Processing conditions | POPs | POPs concentration | Reference |
|---|---|---|---|---|
| *Oils* | | | | |
| Sunflower oil | Heating (frying for 15 min @ 200°C) | 7α-hydroxycampesterol | 0.3 μg/g | Dutta and Appelqvist (1997) |
| | | 7β-hydroxycampesterol | 1.3 μg/g | |
| | | 7-ketocampesterol | 5.9 μg/g | |
| | | Epoxycampesterol[a] | 1.3 μg/g | |
| | | Triolcampesterol | 0.6 μg/g | |
| | | 7α-hydroxysitosterol | 3.8 μg/g | |
| | | 7β-hydroxysitosterol | 7.3 μg/g | |
| | | 7-ketositosterol | 13.1 μg/g | |
| | | Epoxysitosterol[a] | 2.2 μg/g | |
| | | Triolsitosterol | 1.1 μg/g | |
| High-oleic sunflower oil | | 7α-hydroxycampesterol | 1.4 μg/g | |
| | | 7β-hydroxycampesterol | 1.8 μg/g | |
| | | 7-ketocampesterol | 9.2 μg/g | |
| | | Epoxycampesterol[a] | 3.6 μg/g | |
| | | Triolcampesterol | 1.6 μg/g | |
| | | 7α-hydroxysitosterol | 4.7 μg/g | |
| | | 7β-hydroxysitosterol | 9.7 μg/g | |
| | | 7-ketositosterol | 13.5 μg/g | |
| | | Epoxysitosterol[a] | 5.4 μg/g | |
| | | Triolsitosterol | 2.8 μg/g | |
| Olive | • Control<br>• Heating (for 2 hr @ 180 ± 5°C) | 7α-hydroxycampesterol | • 0.2 μg/g oil (control)<br>• 0.6 μg/g oil (for 2 hr @ 180 ± 5°C) | Johnsson and Dutta (2006) |
| | | 7α-hydroxystigmasterol | • 0.6 μg/g oil (control)<br>• 1.2 μg/g oil (for 2 hr @ 180 ± 5°C) | |
| | | 7α-hydroxysitosterol | • 0.3 μg/g oil (control)<br>• 4.7 μg/g oil (for 2 hr @ 180 ± 5°C) | |
| *Oils* | | | | |
| Olive | • Control<br>• Heating (for 2 hr @ 180 ± 5°C) | 7β-hydroxycampesterol | • ND (control)<br>• ND (for 2 hr @ 180 ± 5°C) | Johnsson and Dutta (2006) |
| | | 7β-hydroxystigmasterol | • 0.3 μg/g oil (control)<br>• 0.8 μg/g oil (for 2 hr @ 180 ± 5°C) | |
| | | 7β-hydroxysitosterol | • 0.2 μg/g oil (control)<br>• 3.2 μg/g oil (for 2 hr @ 180 ± 5°C) | |
| | | 24-hydroxystigmasterol | • 0.4 μg/g oil (control)<br>• ND (for 2 hr @ 180 ± 5°C) | |
| | | 24-hydroxycampesterol | • ND (control)<br>• ND (for 2 hr @ 180 ± 5°C) | |
| | | 24-hydroxysitosterol | • ND (control)<br>• ND (for 2 hr @ 180 ± 5°C) | |
| | | 5β,6β-epoxycampesterol | • 0.1 μg/g oil (control)<br>• 0.3 μg/g oil (for 2 hr @ 180 ± 5°C) | |

*Figure 7* *(cont'd)*



**TABLE 2** (Continued)

Phytosterols oxidation products (POPs) concentrations in different food matrices (Borges Botelho et al.)

| Food | Processing conditions | POPs | POPs concentration | Reference |
|---|---|---|---|---|
| | | 5α,6α-epoxycampesterol | • 0.1 μg/g oil (control)<br>• 0.3 μg/g oil (for 2 hr @ 180 ± 5°C) | |
| | | 5β,6β-epoxystigmasterol | • ND (control)<br>• ND (for 2 hr @ 180 ± 5°C) | |
| | | 5α,6α-epoxystigmasterol | • ND (control)<br>• ND (for 2 hr @ 180 ± 5°C) | |
| | | 5β,6β-epoxysitosterol | • ND (control)<br>• ND (for 2 hr @ 180 ± 5°C) | |
| | | 5α,6α-epoxysitosterol | • ND (control)<br>• 0.3 μg/g oil (for 2 hr @ 180 ± 5°C) | |
| | | Campestanetriol | • ND (control)<br>• ND (for 2 hr @ 180 ± 5°C) | |
| | | Stigmastanetriol | • 4.3 μg/g oil (control)<br>• 4.4 μg/g oil (for 2 hr @ 180 ± 5°C) | |
| *Oils* | | | | |
| Olive | • Control<br>• Heating (for 2 hr @ 180 ± 5°C) | Sitostanetriol | • 0.4 μg/g oil (control)<br>• 0.5 μg/g oil (for 2 hr @ 180 ± 5°C) | Johnsson and Dutta (2006) |
| | | 7-ketocampesterol | • ND (control)<br>• ND (for 2 hr @ 180 ± 5°C) | |
| | | 7-ketostigmasterol | • 0.1 μg/g oil (control)<br>• ND (for 2 hr @ 180 ± 5°C) | |
| | | 7-ketositosterol | • 0.8 μg/g oil (control)<br>• 1.6 μg/g oil (for 2 hr @ 180 ± 5°C) | |
| Maize | | 7α-hydroxycampesterol | • 0.1 μg/g oil (control)<br>• 0.7 μg/g oil (for 2 hr @ 180 ± 5°C) | |
| | | 7α-hydroxystigmasterol | • 0.2 μg/g oil (control)<br>• 0.5 μg/g oil (for 2 hr @ 180 ± 5°C) | |
| | | 7α-hydroxysitosterol | • 0.3 μg/g oil (control)<br>• 1.3 μg/g oil (for 2 hr @ 180 ± 5°C) | |
| | | 7β-hydroxycampesterol | • ND (control)<br>• 0.9 μg/g oil (for 2 hr @ 180 ± 5°C) | |
| | | 7β-hydroxystigmasterol | • ND (control)<br>• 0.7 μg/g oil (for 2 hr @ 180 ± 5°C) | |
| | | 7β-hydroxysitosterol | • 0.3 μg/g oil (control)<br>• 2.8 μg/g oil (for 2 hr @ 180 ± 5°C) | |
| | | 24-hydroxystigmasterol | • ND (control)<br>• 0.9 μg/g oil (for 2 hr @ 180 ± 5°C) | |
| | | 24-hydroxycampesterol | • ND (control)<br>• ND (for 2 hr @ 180 ± 5°C) | |
| | | 24-hydroxysitosterol | • ND (control)<br>• ND (for 2 hr @ 180 ± 5°C) | |

*Figure 7* *(cont'd)*



**TABLE 2** (Continued)

Phytosterols oxidation products (POPs) concentrations in different food matrices (Borges Botelho et al.)

| Food | Processing conditions | POPs | POPs concentration | Reference |
|---|---|---|---|---|
| | | 5β,6β-epoxycampesterol | • 0.2 μg/g oil (control)<br>• 0.3 μg/g oil (for 2 hr @ 180 ± 5°C) | |
| *Oils* | | | | |
| Maize | • Control<br>• Heating (for 2 hr @ 180 ± 5°C) | 5α,6α-epoxycampesterol | • 0.2 μg/g oil (control)<br>• 0.2 μg/g oil (for 2 hr @ 180 ± 5°C) | Johnsson and Dutta (2006) |
| | | 5β,6β-epoxystigmasterol | • ND (control)<br>• ND (for 2 hr @ 180 ± 5°C) | |
| | | 5α,6α-epoxystigmasterol | • N (control)<br>• ND (Johnsson and Dutta, p. 180 ± 185 (C) | |
| | | 5β,6β-epoxysitosterol | • 0.6 μg/g oil (control)<br>• 0.5 μg/g oil (for 2 hr @ 180 ± 5°C) | |
| | | 5α,6α-epoxysitosterol | • 0.5 μg/g oil (control)<br>• 0.2 μg/g oil (for 2 hr @ 180 ± 5°C) | |
| | | Campestanetriol | • 0.3 μg/g oil (control)<br>• 0.2 μg/g oil (for 2 hr @ 180 ± 5°C) | |
| | | Stigmastanetriol | • 0.2 μg/g oil (control)<br>• 0.3 μg/g oil (for 2 hr @ 180 ± 5°C) | |
| | | Sitostanetriol | • 0.3 μg/g oil (control)<br>• 0.8 μg/g oil (for 2 hr @ 180 ± 5°C) | |
| | | 7-ketocampesterol | • ND (control)<br>• 0.4 μg/g oil (for 2 hr @ 180 ± 5°C) | |
| | | 7-ketostigmasterol | • 0.1 μg/g oil (control)<br>• ND (for 2 hr @ 180 ± 5°C) | |
| | | 7-ketositosterol | • 1.0 μg/g oil (control)<br>• 1.5 μg/g oil (for 2 hr @ 180 ± 5°C) | |
| *Eggs* | | | | |
| Egg | Heating (shallow-frying with liquid margarine) | Total POPs | 0.29 ± 0.09 mg per portion size | Lin et al. (2016) |
| | | 7-hydroxysitosterol[b] | 0.031 mg/portion of food | Lin et al., (2018) |
| | | 7-ketositosterol | 0.100 mg/portion of food | |
| | | Epoxysitosterol[a] | 0.010 mg/portion of food | |
| | | Triolsitosterol | 0.012 mg/portion of food | |
| | | 7-hydroxycampesterol[b] | 0.009 mg/portion of food | |
| | | 7-ketocampesterol | 0.020 mg/portion of food | |
| | | Epoxycampesterol[a] | 0.007 mg/portion of food | |
| | | Triolcampesterol | 0.002 mg/portion of food | |
| | N/A | Total POPs | 0.3 mg POPs/100 g ingredient | Menéndez-Carreño, Knol, and Janssen (2016) |
| *Dairy* | | | | |
| Brick margarine | Heating (16 min @ 210°C) | Sitosterol-POP | 4.3 ± 0.3 mg/100 g fat | Lin et al. (2017) |
| | | Campesterol-POP | 3.2 ± 0.3 mg/100 g fat | |

*Figure 7* *(cont'd)*



**TABLE 2** (Continued)

Phytosterols oxidation products (POPs) concentrations in different food matrices (Borges Botelho et al.)

| Food | Processing conditions | POPs | POPs concentration | Reference |
|---|---|---|---|---|
| Liquid margarine | | Sitosterol-POP | 6.4 ± 0.03 mg/100 g fat | |
| | | Campesterol-POP | 3.2 ± 0.2 mg/100 g fat | |
| *Meat and poultry* | | | | |
| Chicken | Heating (stir-fried with liquid margarine) | Total POPs | 0.53 ± 0.15 mg per portion size | Lin et al. (2016) |
| | | 7-hydroxysitosterol[b] | 0.122 mg/portion of food | Lin et al. (2018) |
| | | 7-ketositosterol | 0.101 mg/portion of food | |
| | | Epoxysitosterol[a] | 0.221 mg/portion of food | |
| | | Triolsitosterol | 0.003 mg/portion of food | |
| | | 7-hydroxycampesterol[b] | 0.050 mg/portion of food | |
| | | 7-ketocampesterol | 0.024 mg/portion of food | |
| | | Epoxycampesterol[a] | 0.085 mg/portion of food | |
| | | Triolcampesterol | 0.002 mg/portion of food | |
| Pork fillet | Heating (shallow-frying with liquid margarine) | Total POPs | 0.05 ± 0.03 mg per portion size | Lin et al. (2016) |
| | | 7-hydroxysitosterol[b] | 0.120 mg/portion of food | Lin et al. (2018) |
| | | 7-ketositosterol | 0.311 mg/portion of food | |
| | | Epoxysitosterol[a] | 0.440 mg/portion of food | |
| | | Triolsitosterol | 0.004 mg/portion of food | |
| | | 7-hydroxycampesterol[b] | 0.040 mg/portion of food | |
| | | 7-ketocampesterol | 0.083 mg/portion of food | |
| | | Epoxycampesterol[a] | 0.113 mg/portion of food | |
| | | Triolcampesterol | 0.003 mg/portion of food | |
| Steak (beef) | | Total POPs | 0.97 ± 0.39 mg per portion size | Lin et al. (2016) |
| | | 7-hydroxysitosterol[b] | 0.120 mg/portion of food | Lin et al. (2018) |
| | | 7-ketositosterol | 0.102 mg/portion of food | |
| | | Epoxysitosterol[a] | 0.450 mg/portion of food | |
| | | Triolsitosterol | 0.003 mg/portion of food | |
| | | 7-hydroxycampesterol[b] | 0.065 mg/portion of food | |
| | | 7-ketocampesterol | 0.024 mg/portion of food | |
| | | Epoxycampesterol[a] | 0.157 mg/portion of food | |
| | | Triolcampesterol | 0.003 mg/portion of food | |
| *Meat and poultry* | | | | |
| Minced meat | Heating (shallow-frying with liquid margarine) | Total POPs | 0.74 ± 0.46 mg per portion size | Lin et al. (2016) |
| | | 7-hydroxysitosterol[b] | 0.216 mg/portion of food | Lin et al. (2018) |
| | | 7-ketositosterol | 0.001 mg/portion of food | |
| | | Epoxysitosterol[a] | 0.373 mg/portion of food | |
| | | Triolsitosterol | 0.003 mg/portion of food | |
| | | 7-hydroxycampesterol[b] | 0.082 mg/portion of food | |
| | | 7-ketocampesterol | 0.014 mg/portion of food | |
| | | Epoxycampesterol[a] | 0.172 mg/portion of food | |
| | | Triolcampesterol | 0.003 mg/portion of food | |
| Beef | Heating (stewing with liquid margarine) | Total POPs | 0.75 ± 0.25 mg per portion size | Lin et al. (2016) |
| | | 7-hydroxysitosterol[b] | 0.101 mg/portion of food | Lin et al. (2018) |
| | | 7-ketositosterol | 0.243 mg/portion of food | |
| | | Epoxysitosterol[a] | 0.141 mg/portion of food | |
| | | Triolsitosterol | 0.003 mg/portion of food | |

*Figure 7* (cont'd)



**TABLE 2** (Continued)

Phytosterols oxidation products (POPs) concentrations in different food matrices (Borges Botelho et al.)

| Food | Processing conditions | POPs | POPs concentration | Reference |
|---|---|---|---|---|
| | | 7-hydroxycampesterol[b] | 0.034 mg/portion of food | |
| | | 7-ketocampesterol | 0.014 mg/portion of food | |
| | | Epoxycampesterol[a] | 0.030 mg/portion of food | |
| | | Triolcampesterol | 0.003 mg/portion of food | |
| | Heating (roasting with liquid margarine) | Total POPs | 0.60 ± 0.18 mg per portion size | Lin et al. (2016) |
| | | 7-hydroxysitosterol[b] | 0.033 mg/portion of food | Lin et al. (2018) |
| | | 7-ketositosterol | 0.731 mg/portion of food | |
| | | Epoxysitosterol[a] | 0.085 mg/portion of food | |
| | | Triolsitosterol | 0.006 mg/portion of food | |
| | | 7-hydroxycampesterol[b] | 0.012 mg/portion of food | |
| | | 7-ketocampesterol | 0.142 mg/portion of food | |
| | | Epoxycampesterol[a] | 0.017 mg/portion of food | |
| | | Triolcampesterol | 0.004 mg/portion of food | |
| *Meat and poultry* | | | | |
| Meat (steak, roast beef, stew, chicken, pork, and minced meat) | N/A | Total POPs | 0.04–0.9 mg POPs/100 g of ingredients | Menéndez-Carreño, Knol, and Janssen (2016) |
| *Seafood* | | | | |
| Codfish | Heating (shallow-frying with liquid margarine) | Total POPs | 0.21 ± 0.05 mg per portion size | Lin et al. (2016) |
| | | 7-hydroxysitosterol[b] | 0.142 mg/portion of food | Lin et al. (2018) |
| | | 7-ketositosterol | 0.256 mg/portion of food | |
| | | Epoxysitosterol[a] | 0.264 mg/portion of food | |
| | | Triolsitosterol | 0.003 mg/portion of food | |
| | | 7-hydroxycampesterol[b] | 0.045 mg/portion of food | |
| | | 7-ketocampesterol | 0.071 mg/portion of food | |
| | | Epoxycampesterol[a] | 0.101 mg/portion of food | |
| | | Triolcampesterol | 0.003 mg/portion of food | |
| | Heating (microwave with liquid margarine) | Total POPs | 0.05 ± 0.01 mg per portion size | Lin et al. (2016) |
| | | 7-hydroxysitosterol[b] | 0.026 mg/portion of food | Lin et al. (2018) |
| | | 7-ketositosterol | 0.209 mg/portion of food | |
| | | Epoxysitosterol[a] | 0.083 mg/portion of food | |
| | | Triolsitosterol | 0.009 mg/portion of food | |
| | | 7-hydroxycampesterol[b] | 0.009 mg/portion of food | |
| | | 7-ketocampesterol | 0.032 mg/portion of food | |
| | | Epoxycampesterol[a] | 0.004 mg/portion of food | |
| | | Triolcampesterol | 0.003 mg/portion of food | |
| Fish fingers | Heating (shallow-frying with liquid margarine) | Total POPs | 1.11 ± 0.46 mg per portion size | Lin et al. (2016) |
| | | 7-hydroxysitosterol[b] | 0.303 mg/portion of food | Lin et al. (2018) |
| | | 7-ketositosterol | 0.131 mg/portion of food | |
| | | Epoxysitosterol[a] | 0.943 mg/portion of food | |
| | | Triolsitosterol | 0.005 mg/portion of food | |
| *Seafood* | | | | |
| Fish fingers | Heating (shallow-frying with liquid margarine) | 7-hydroxycampesterol[b] | 0.163 mg/portion of food | Lin et al. (2018) |
| | | 7-ketocampesterol | 0.048 mg/portion of food | |
| | | Epoxycampesterol[a] | 0.316 mg/portion of food | |
| | | Triolcampesterol | 0.003 mg/portion of food | |

*Figure 7* *(cont'd)*



**TABLE 2** (Continued)

| Phytosterols oxidation products (POPs) concentrations in different food matrices (Borges Botelho et al.) | | | | |
|---|---|---|---|---|
| **Food** | **Processing conditions** | **POPs** | **POPs concentration** | **Reference** |
| Salmon | | Total POPs | 0.57 ± 0.15 mg per portion size | Lin et al. (2016) |
| | | 7-hydroxysitosterol[b] | 0.203 mg/portion of food | Lin et al. (2018) |
| | | 7-ketositosterol | 0.138 mg/portion of food | |
| | | Epoxysitosterol[a] | 0.504 mg/portion of food | |
| | | Triolsitosterol | 0.003 mg/portion of food | |
| | | 7-hydroxycampesterol[b] | 0.111 mg/portion of food | |
| | | 7-ketocampesterol | 0.172 mg/portion of food | |
| | | Epoxycampesterol[a] | 0.032 mg/portion of food | |
| | | Triolcampesterol | 0.003 mg/portion of food | |
| Fish (salmon, shallow-fried cod, microwaved cod, and fish sticks) | N/A | Total POPs | 0.04–0.8 mg POPs/100 g of ingredients | Lin et al. (2018) |
| *Others* | | | | |
| Wafer biscuit | N/A | Total POPs | 3.91–27.81 mg/kg | Hu et al. (2018) |
| Soda biscuit | | | 4.10–6.30 mg/kg | |
| Cookies | | | 3.88–8.16 mg/kg | |
| Biscuit | | | 2.84–7.90 mg/kg | |
| Sandwich biscuit | | | 3.19–9.00 mg/kg | |
| Egg roll | | | 4.11–12.04 mg/kg | |
| Egg pie | | | 1.14–8.19 mg/kg | |
| Caramel treats | | | 4.96–9.79 mg/kg | |
| Cake | | | 3.50–18.17 mg/kg | |
| Egg tart | | | 1.07–6.16 mg/kg | |
| Staple bread | | | 1.05–7.62 mg/kg | |
| *Others* | | | | |
| Fancy bread | N/A | Total POPs | 2.14–8.00 mg/kg | Hu et al. (2018) |
| Recuperation bread | | | 0.37–5.43 mg/kg | |
| French fries | • Control<br>• Heating (oven for 15 min @ 225°C) | 5α,6α-epoxycampesterol | • ND (control)<br>• 5.2 ± 0.4 mg/100 g fat (heated) | Derewiaka and Obiedziński (2012) |
| | | 5β,6β-epoxycampesterol | • ND (control)<br>• 4.7 ± 0.3 mg/100 g fat (heated) | |
| Green beans | Heating (stir-fried with liquid margarine) | Total POPs | 0.55 ± 0.08 mg per portion size | Lin et al. (2016) |
| | | 7-hydroxysitosterol[b] | 0.030 mg/portion of food | Lin et al. (2018) |
| | | 7-ketositosterol | 0.091 mg/portion of food | |
| | | Epoxysitosterol[a] | 0.060 mg/portion of food | |
| | | Triolsitosterol | 0.003 mg/portion of food | |
| | | 7-hydroxycampesterol[b] | 0.012 mg/portion of food | |
| | | 7-ketocampesterol | 0.021 mg/portion of food | |
| | | Epoxycampesterol[a] | 0.009 mg/portion of food | |
| | | Triolcampesterol | 0.003 mg/portion of food | |
| Cabbage | | Total POPs | 0.67 ± 0.11 mg per portion size | Lin et al. (2016) |
| | | 7-hydroxysitosterol[b] | 0.030 mg/portion of food | Lin et al. (2018) |

*Figure 7* *(cont'd)*



**TABLE 2** (Continued)

Phytosterols oxidation products (POPs) concentrations in different food matrices (Borges Botelho et al.)

| Food | Processing conditions | POPs | POPs concentration | Reference |
|---|---|---|---|---|
| | | 7-ketositosterol | 0.052 mg/portion of food | |
| | | Epoxysitosterol[a] | 0.050 mg/portion of food | |
| | | Triolsitosterol | 0.001 mg/portion of food | |
| | | 7-hydroxycampesterol[b] | 0.014 mg/portion of food | |
| | | 7-ketocampesterol | 0.012 mg/portion of food | |
| | | Epoxycampesterol[a] | 0.026 mg/portion of food | |
| | | Triolcampesterol | 0.002 mg/portion of food | |
| Onions | Heating (shallow-fried with liquid margarine) | Total POPs | 0.30 ± 0.04 mg per portion size | Lin et al. (2016) |
| | | 7-hydroxysitosterol[b] | 0.027 mg/portion of food | Lin et al. (2018) |
| | | 7-ketositosterol | 0.107 mg/portion of food | |
| | | Epoxysitosterol[a] | 0.058 mg/portion of food | |
| | | Triolsitosterol | 0.003 mg/portion of food | |
| *Others* | | | | |
| Onions | Heating (shallow-fried with liquid margarine) | 7-hydroxycampesterol[b] | 0.008 mg/portion of food | Lin et al. (2018) |
| | | 7-ketocampesterol | 0.021 mg/portion of food | |
| | | Epoxycampesterol[a] | 0.015 mg/portion of food | |
| | | Triolcampesterol | 0.001 mg/portion of food | |
| Potatoes | | Total POPs | 0.99 ± 0.14 mg per portion size | Lin et al. (2016) |
| | | 7-hydroxysitosterol[b] | 0.690 mg/portion of food | Lin et al. (2018) |
| | | 7-ketositosterol | 0.236 mg/portion of food | |
| | | Epoxysitosterol[a] | 0.504 mg/portion of food | |
| | | Triolsitosterol | 0.009 mg/portion of food | |
| | | 7-hydroxycampesterol[b] | 0.317 mg/portion of food | |
| | | 7-ketocampesterol | 0.084 mg/portion of food | |
| | | Epoxycampesterol[a] | 0.534 mg/portion of food | |
| | | Triolcampesterol | 0.004 mg/portion of food | |
| Vegetables and potato (onion, cabbage, green beans, and potato) | N/A | Total POPs | 0.4–0.6 mg POPs/100 g of ingredients | Menéndez-Carreño et al. (2016) |
| French fries | • Control<br>• Heating (oven for 15 min @ 225° C) | 7-ketocampesterol | • ND (control)<br>14.2 ± 0.8 mg/100 g fat (heated) | Derewiaka and Obiedziński (2012) |
| | | 5α,6α-epoxysitosterol | • ND (control)<br>9.5 ± 0.9 mg/100 g fat (heated) | |
| | | 7-ketositosterol | • ND (control)<br>35.2 ± 3.9 mg/100 g fat (heated) | |
| Chocolate bar | Storage (20° and 30°C for 5 months) | 7α-hydroxycampesterol | 77.85 ± 1.33 g/bar | Borges Botelho et al. (2014) |
| | | 7α-hydroxystigmasterol | 72.79 ± 2.56 g/bar | |
| | | 7β-hydroxystigmasterol | 24.93 ± 0.60 g/bar | |
| | | 7-ketocampesterol | 58.83 ± 3.61 g/bar | |
| *Others* | | | | |
| Chocolate bar | Storage (20° and 30°C for 5 months) | Sitostanetriol | 165.84 ± 7.79 g/bar | Borges Botelho et al. (2014) |
| | | 6-ketositosterol | 67.13 ± 1.27 g/bar | |
| | | 7-ketositsterol | 66.26 ± 4.46 g/bar | |
| | | 7α-hydroxycampesterol | 109.48 ± 3.95 g/bar | |
| | | 7α-hydroxystigmasterol | 92.32 ± 2.12 g/bar | |
| | | 7β-hydroxystigmasterol | 52.33 ± 3.09 g/bar | |

*Figure 7* *(cont'd)*



**TABLE 2** (Continued)

| Phytosterols oxidation products (POPs) concentrations in different food matrices (Borges Botelho et al.) | | | | |
|---|---|---|---|---|
| **Food** | **Processing conditions** | **POPs** | **POPs concentration** | **Reference** |
| Chocolate bar + palm oil | | 7α-hydroxysitosterol | 173.19 ± 3.02 g/bar | |
| | | α-epoxysitosterol | 140.36 ± 4.94 g/bar | |
| | | 7-ketocampesterol | 211.57 ± 15.12 g/bar | |
| | | 6β-hydroxycampesterol | 181.08 ± 13.28 g/bar | |
| | | Stigmastentriol | 45.54 ± 0.31 g/bar | |
| | | Sitostanetriol | 717.56 ± 108.16 g/bar | |
| | | 6-ketositosterol | 228.75 ± 22.70 g/bar | |
| | | 7-ketositsterol | 179.97 ± 34.37 g/bar | |
| Chocolate bar + palm oil + ascorbic Acid and α-tocopherol) | | 7α-hydroxycampesterol | 114.28 ± 2.24 g/bar | |
| | | 7α-hydroxystigmasterol | 97.03 ± 0.62 g/bar | |
| | | 7β-hydroxystigmasterol | 58.97 ± 2.75 g/bar | |
| | | 7α-hydroxysitosterol | 178.32 ± 3.30 g/bar | |
| | | α-epoxysitosterol | 172.77 ± 15.23 g/bar | |
| | | 7-ketocampesterol | 198.59 ± 8.62 g/bar | |
| | | 6β-hydroxucampesterol | 252.94 ± 55.37 g/bar | |
| | | Stigmastentriol | 54.35 ± 0.89 g/bar | |
| | | Sitostanetriol | 933.83 ± 109.27 g/bar | |
| | | 6-ketositosterol | 257.90 ± 35.85 g/bar | |
| | | 7-ketositsterol | 160.22 ± 1.57 g/bar | |
| *Others* | | | | |
| Tablet supplements manufactured in Finland | • Anti K-steroli<br>• Tri Tolosen Kasvisteroli<br>• Kolestop | 7α-hydroxycampesterol | • 0.2 mg/100 g sample (anti K-steroli)<br>• 0.2 mg/100 g sample (tri Tolosin Kasvisteroli)<br>• 0.3 mg/100 g sample (Kolestop) | Dutta (1999) |
| | | 7β-hydroxycampesterol | • 0.2 mg/100 g sample (anti K-steroli)<br>• 0.2 mg/100 g sample (tri Tolosin Kasvisteroli)<br>• 0.2 mg/100 g sample (Kolestop) | |
| | | 7-ketocampesterol | • 0.2 mg/100 g sample (anti K-steroli)<br>• 0.3 mg/100 g sample (tri Tolosin Kasvisteroli)<br>• 0.2 mg/100 g sample (Kolestop) | |
| | | 5α,6α-epoxycampesterol | • Tr mg/100 g sample (anti K-steroli)<br>• 0.6 mg/100 g sample (tri Tolosin Kasvisteroli)<br>• 0.3 mg/100 g sample (Kolestop) | |
| | | 5β,6β-epoxycampesterol | • 0.2 mg/100 g (anti K-steroli)<br>• 2.5 mg/100 g (tri Tolosin Kasvisteroli)<br>• 0.8 mg/100 g (Kolestop) | |
| | | Triolcampesterol | • ND mg/100 g (anti K-steroli)<br>• ND mg/100 g (tri Tolosin Kasvisteroli)<br>• ND mg/100 g (Kolestop) | |

*Figure 7* *(cont'd)*



**TABLE 2** (Continued)

Phytosterols oxidation products (POPs) concentrations in different food matrices (Borges Botelho et al.)

| Food | Processing conditions | POPs | POPs concentration | Reference |
|---|---|---|---|---|
| *Others* | | | | |
| Tablet supplements manufactured in Finland | • Anti K-steroli<br>• Tri Tolosen Kasvisteroli<br>• Kolestop | 7α-hydroxysitosterol | • 0.8 mg/100 g (anti K-steroli)<br>• 1.8 mg/100 g (tri Tolosin Kasvisteroli)<br>• 2.0 mg/100 g (Kolestop) | Dutta (1999) |
| | | 7β-hydroxysitosterol | • 1.8 mg/100 g (anti K-steroli)<br>• 0.8 mg/100 g (tri Tolosin Kasvisteroli)<br>• 3.8 mg/100 g (Kolestop) | |
| | | 7-ketositosterol | • 2.7 mg/100 g (anti K-steroli)<br>• 4.0 mg/100 g (tri Tolosin Kasvisteroli)<br>• 5.0 mg/100 g (Kolestop) | |
| | | 5α,6α-epoxysitosterol | • 1.6 mg/100 g sample (anti K-steroli)<br>• 1.7 mg/100 g sample (tri Tolosin Kasvisteroli)<br>• 3.1 mg/100 g sample (Kolestop) | |
| | | 5β,6β-epoxysitosterol | • 2.8 mg/100 g sample (anti K-steroli)<br>• 9.1 mg/100 g sample (tri Tolosin Kasvisteroli)<br>• 9.9 mg/100 g sample (Kolestop) | |
| | | Triolsitosterol | • 0.2 mg/100 g sample (anti K-steroli)<br>• 0.3 mg/100 g sample (tri Tolosin Kasvisteroli)<br>• 0.2 mg/100 g sample (Kolestop) | |
| *Others* | | | | |
| Potato chips fried in high-sunflower oil | Storage (25 weeks) | 7α-hydroxycampesterol | 0.7 μg/g | Dutta (1997) |
| | | 7β-hydroxycampesterol | 1.5 μg/g | |
| | | 7-ketocampesterol | 6.2 μg/g | |
| | | Epoxycampesterol[a] | 3.5 μg/g | |
| | | Triolcampesterol | 1.8 μg/g | |
| | | 7α-hydroxysitosterol | 5.3 μg/g | |
| | | 7β-hydroxysitosterol | 11.5 μg/g | |
| | | 7-ketositosterol | 18.1 μg/g | |
| | | Epoxysitosterol[a] | 6.3 μg/g | |
| | | Triolsitosterol | 3.6 μg/g | |
| Potato chips fried in sunflower oil | | 7α-hydroxycampesterol | 0.6 μg/g | |
| | | 7β-hydroxycampesterol | 1.5 μg/g | |
| | | 7-ketocampesterol | 6.2 μg/g | |
| | | Epoxycampesterol[a] | 5.3 μg/g | |
| | | Triolcampesterol | 2.6 μg/g | |
| | | 7α-hydroxysitosterol | 6.6 μg/g | |
| | | 7β-hydroxysitosterol | 8.8 μg/g | |
| | | 7-ketositosterol | 15.7 μg/g | |

*Figure 7* *(cont'd)*



**TABLE 2** (Continued)

Phytosterols oxidation products (POPs) concentrations in different food matrices (Borges Botelho et al.)

| Food | Processing conditions | POPs | POPs concentration | Reference |
|---|---|---|---|---|
| Margarine | • Control<br>• Heating (microwave, bottle @ 800 W for 4 min)<br>• Heating (Min et al., p. 200 (C for 209 min)<br>• Heating (oven, bottle @ 200 °C for 20 min)<br>• Heating (oven, casserole @ 200°C for 20 min) | 5β,6β-epoxycampesterol | • 4.4 ± 0.1 mg/kg (control)<br>• 31.0 ± 1.5 mg/kg (microwave, bottle @ 800 W for 4 min)<br>• 43.8 ± 0.8 mg/kg (Min et al., p. 200 (C for 209 min)<br>• 140.0 ± 1.9 mg/kg (oven, bottle @ 200°C for 20 min)<br>• 227.1 ± 6.1 mg/kg (oven, casserole @ 200°C for 20 min) | Scholz (2016) |
| *Others* | | | | |
| Margarine | • Control<br>• Heating (microwave, bottle @ 800 W for 4 min)<br>• Heating (Min et al., p. 200 (C for 209 min)<br>• Heating (oven, bottle @ 200° C for 20 min)<br>• Heating (oven, casserole @ 200°C for 20 min) | 5β,6β-epoxysitosterol | • 14.8 ± 0.7 mg/kg (control)<br>• 152.4 ± 5.3 mg/kg (microwave, bottle @ 800 W for 4 min)<br>• 213.6 ± 9.1 mg/kg (Min et al., p. 200 (C for 209 min)<br>• 641.0 ± 11.5 mg/kg (oven, bottle @ 200°C for 20 min)<br>• 1,030.6 ± 11.5 mg/kg (oven, casserole @ 200°C for 20 min) | Scholz (2016) |
| | | 5α,6α-epoxycampesterol | • 3.8 ± 0.3 mg/kg (control)<br>• 15.9 ± 0.2 mg/kg (microwave, bottle @ 800 W for 4 min)<br>• 20.6 ± 0.3 mg/kg (Min et al., p. 200 (C for 209 min)<br>• 59.9 ± 1.7 mg/kg (oven, bottle @ 200°C for 20 min)<br>• 115.5 ± 2.7 mg/kg (oven, casserole @ 200°C for 20 min) | |
| | | 5α,6α-epoxysitosterol | • 18.7 ± 1.8 mg/kg (control)<br>• 69.6 ± 3.1 mg/kg (microwave, bottle @ 800 W for 4 min)<br>• 100.8 ± 5.0 mg/kg (Min et al., p. 200 (C for 209 min)<br>• 289.2 ± 15.8 mg/kg (oven, bottle @ 200°C for 20 min)<br>• 508.6 ± 4.8 mg/kg (oven, casserole @ 200°C for 20 min) | |
| *Others* | | | | |
| Margarine | • Control<br>• Heating (microwave, bottle @ 800 W for 4 min)<br>• Heating (Min et al., p. 200 (C for 209 min)<br>• Heating (oven, bottle @ 200° C for 20 min)<br>• Heating (oven, casserole @ 200°C for 20 min) | 7α-hydroxycampesterol | • 2.9 ± 0.3 mg/kg (control)<br>• 26.7 ± 1.0 mg/kg (microwave, bottle @ 800 W for 4 min)<br>• 42.8 ± 1.7 mg/kg (Min et al., p. 200 (C for 209 min)<br>• 97.0 ± 4.5 mg/kg (oven, bottle @ 200°C for 20 min)<br>• 207.0 ± 8.1 mg/kg (oven, casserole @ 200°C for 20 min) | Scholz (2016) |

*Figure 7* *(cont'd)*



**TABLE 2** (Continued)

Phytosterols oxidation products (POPs) concentrations in different food matrices (Borges Botelho et al.)

| Food | Processing conditions | POPs | POPs concentration | Reference |
|---|---|---|---|---|
| | | 7α-hydroxysitosterol | • 3.1 ± 0.2 mg/kg (control)<br>• 90.0 ± 2.5 mg/kg (microwave, bottle @ 800 W for 4 min)<br>• 155.8 ± 3.6 mg/kg (Min et al., p. 200 (C for 209 min)<br>• 338.0 ± 3.5 mg/kg (oven, bottle @ 200°C for 20 min)<br>• 704.5 ± 22.5 mg/kg (oven, casserole @ 200°C for 20 min) | |
| | | 7β-hydroxycampesterol | • 1.4 ± 0.0 mg/kg (control)<br>• 23.0 ± 1.1 mg/kg (microwave, bottle @ 800 W for 4 min)<br>• 33.0 ± 1.7 mg/kg (Min et al., p. 200 (C for 209 min)<br>• 89.7 ± 4.1 mg/kg (oven, bottle @ 200°C for 20 min)<br>• 171.9 ± 3.4 mg/kg (oven, casserole @ 200°C for 20 min) | |
| *Others* | | | | |
| Margarine | • Control<br>• Heating (microwave, bottle @ 800 W for 4 min)<br>• Heating (Min et al., p. 200 (C for 209 min)<br>• Heating (oven, bottle @ 200°C for 20 min)<br>• Heating (oven, casserole @ 200°C for 20 min) | 7β-hydroxysitosterol | • 4.7 ± 0.4 mg/kg (control)<br>• 103.5 ± 3.8 mg/kg (microwave, bottle @ 800 W for 4 min)<br>• 167.6 ± 6.3 mg/kg (Min et al., p. 200 (C for 209 min)<br>• 354.6 ± 8.5 mg/kg (oven, bottle @ 200°C for 20 min)<br>• 673.5 ± 15.4 mg/kg (oven, casserole @ 200°C for 20 min) | Scholz (2016) |
| | | 6α-hydroxysitostanol | • <0.33 μg/mL of injection volume (control)<br>• 24.8 ± 2.8 mg/kg (microwave, bottle @ 800 W for 4 min)<br>• 49.6 ± 3.5 mg/kg (Min et al., p. 200 (C for 209 min)<br>• 44.3 ± 3.6 mg/kg (oven, bottle @ 200°C for 20 min)<br>• 77.7 ± 2.3 mg/kg (oven, casserole @ 200°C for 20 min) | |
| | | 7-ketocampesterol | • 10.2 ± 1.0 mg/kg (control)<br>• 40.4 ± 0.8 mg/kg (microwave, bottle @ 800 W for 4 min)<br>• 46.4 ± 1.2 mg/kg (Min et al., p. 200 (C for 209 min)<br>• 157.9 ± 3.3 mg/kg (oven, bottle @ 200°C for 20 min)<br>• 257.1 ± 8.9 mg/kg (oven, casserole @ 200°C for 20 min) | |

*Figure 7* *(cont'd)*



**TABLE 2** (Continued)

Phytosterols oxidation products (POPs) concentrations in different food matrices (Borges Botelho et al.)

| Food | Processing conditions | POPs | POPs concentration | Reference |
|---|---|---|---|---|
| *Others* | | | | |
| Margarine | • Control<br>• Heating (microwave, bottle @ 800 W for 4 min)<br>• Heating (Min et al., p. 200 (C for 209 min)<br>• Heating (oven, bottle @ 200 °C for 20 min)<br>• Heating (oven, casserole @ 200°C for 20 min) | 7-ketositosterol | • 28.9 ± 0.7 mg/kg (control)<br>• 203.0 ± 2.8 mg/kg (microwave, bottle @ 800 W for 4 min)<br>• 204.9 ± 5.4 mg/kg (Min et al., p. 200 (C for 209 min)<br>• 691.0 ± 13.0 mg/kg (oven, bottle @ 200°C for 20 min)<br>• 1,135.9.4 ± 40.5 mg/kg (oven, casserole @ 200°C for 20 min) | Scholz (2016) |
| Potato chips fried in sunflower oil | Storage (25 weeks) | Epoxysitosterol[a] | 4.6 μg/g | Dutta (1997) |
| | | Triolsitosterol | 0.8 μg/g | |
| Potato chips fried in palm oil | | 7α-hydroxycampesterol | 0.1 μg/g | |
| | | 7β-hydroxycampesterol | 0.2 μg/g | |
| | | 7-ketocampesterol | 0.7 μg/g | |
| | | Epoxycampesterol[a] | 0.2 μg/g | |
| | | Triolcampesterol | ND | |
| | | 7α-hydroxysitosterol | 1.2 μg/g | |
| | | 7β-hydroxysitosterol | 1.4 μg/g | |
| | | 7-ketositosterol | 2.0 μg/g | |
| | | Epoxysitosterol[a] | 1.9 μg/g | |
| | | Triolsitosterol | 0.9 μg/g | |
| *Bakery products* | | | | |
| Sponge cake, banana bread, muffins, and cookies | N/A | Total POPs | 0.2–0.7 mg POPs/100 g of ingredients | Menéndez-Carreño et al. (2016) |

*Note*: Results are mean values ± *SD*.
Abbreviations: N/A, not applicable; ND, not detected.
[a]Includes both 5α,6α-Epoxysterols and 5β,6β-Epoxysterols.
[b]Includes both 7α-hydroxysterols and 7β-hydroxysterols.

content. The animal sterols, including cholesterol, range from 12.0 to 16.6 mg/100 mL and 0.4–5.47 mg/100 mL in human milk and IF, respectively. The phytosterols content is 0.02 mg/100 mL in human milk versus 2.45–5.07 mg/100 mL in IF, where the main phytosterols are β-sitosterol and stigmasterol (Hamdan et al., 2017). IFs are manufactured to mimic the human milk as close as possible, but many differences remain, bringing up a nutritional concern because of its effect on the baby's health. For example, fat in human milk makes up 50% of the total energy: in order to match that percentage, Ifs need to be supplemented with external fat sources (Chen et al., 2019; Hageman, Danielsen, Nieuwenhuizen, Feitsma, & Dalsgaard, 2019). Other components naturally present in human milk (hormones, vitamins, and essential fatty acids) are also supplemented in IFs to achieve the correct infant dietary requirements (Chen et al., 2019). An interesting case is represented by long chain fatty acids (arachidonic acid, eicosapentanoic acid, and docosahexaenoic acid), which play a crucial role in cognitive and retinal development. IF with a fat composition that comes only from vegetable oil has higher levels of monounsaturated fatty acids and lower levels of medium chain fatty acids compared to human milk (Hageman et al., 2019). Recently, long chain polyunsaturated fatty acids, otherwise present in human milk, have been recently added to IFs as well (Chen et al., 2019; Uauy & Dangour, 2009).

As it is stated above, there is significant evidence of the abundant presence of PS in IF, however, little is known about the effects of processing technologies on its oxidation (García-Llatas et al., 2008; Lagarda et al., 2006). The POP content in IF can be seen in Table 4. Because of the high lipid content in IF lipid due to fat supplementation with vegetable oils, oxidation is likely to occur. Few studies have investigated the formation of POPs within IF. Boatright and Crum (2016) tested three different infant formulations commonly found in

*Figure 7* *(cont'd)*



**TABLE 3** Phytosterols content reported in infant formula

| Phytosterols concentrations in infant formula | | | |
|---|---|---|---|
| Type | Phytosterol | Concentration | Reference |
| Powdered | Brassicasterol | 2.81 ± 0.08 mg/L | Hamdan et al. (2017) |
| | Stigmasterol | 5.04 ± 0.36 mg/L | |
| | β-sitosterol | 30.28 ± 0.23 mg/L | |
| | Campesterol | 13.80 ± 0.17 mg/L | |
| | Sitostanol | 0.98 ± 0.12 mg/L | |
| | Brassicasterol | 0.39 ± 0.01 mg/100 mL of reconstituted IF | Hamdan et al. (2018) |
| | Campesterol | 2.07 ± 0.02 mg/100 mL of reconstituted IF | |
| | Stigmasterol | 0.34 ± 0.06 mg/100 mL of reconstituted IF | |
| | β-sitosterol | 5.72 ± 0.48 mg/100 mL of reconstituted IF | |
| | Sitosterol | 350–1,480 mg/g lipid | Zunin et al. (1998) |
| Liquid | Brassicasterol | 0.18 ± 0.02 mg/100 g | García-Llatas et al. (2008) |
| | Campesterol | 1.18 ± 0.09 mg/100 g | |
| | Stigmasterol | 0.37 ± 0.05 mg/100 g | |
| | β-sitosterol | 3.47 ± 0.37 mg/100 g | |

the store and found that hydrogen peroxide, which is one of the ROS that can lead to lipid oxidation, was generated when preparing the formula according to the manufacturer's directions. Since the formation did not occur until after mixing with water, they concluded that the hydrogen peroxide was generated via a redox-cycling reaction from the initial ingredients inside the IF. This type of study emphasizes the need of perform more research focused on the importance of IF and the effects of its manufacturing in the formation of POPs.

New studies have started questioning the high PS content in IF, and hence, its potential oxidation process occurring during the infant formula manufacturing which generates POPs. Berger et al. (2004) recommended that children under five should not be given phytosterols since they should have high cholesterol in their diets instead (Lemaire et al., 2018). Therefore, a question that arises is: Do we need to modify the IF recipe or the manufacturing process to dampen its lipid oxidative load?

## 7 | PROCESSING TECHNOLOGY RELATED TO IF

Like most foods nowadays, IF undergoes rigorous processing prior to commercialization in order to guarantee the safety of the final product. IF is usually made by modifying cow's milk, with a variation in the whey-to-casein ratio (70/30). Whey proteins are predominant in breast milk (60% whey and 40% casein) and are believed to be more easily digested. Other substances that can be added are prebiotics (to aid digestion) and nucleotides. The former is normally found in breast milk and promotes brain and eye development (Desic & Birlouez-Aragon, 2011; Traves, 2015). In general, the manufacturing process of IF involves a combination of different temperatures, pressures, and times, with a wide range varying between 60 and 200°C, 0.8–20 MPa, and 30 s to 6 min, respectively. All these differences in the manufacturing procedures promotes the oxidation of the phytosterols present in the food matrix and produce potentially toxic compounds, such as POPs.

A general IF manufacturing process is shown in Figure 3. The main process steps are: mixing, evaporation, and drying (Jiang & Guo, 2014). Temperature and pressure are the major parameters affecting the evaporation and drying processing steps. Evaporation, a critical process step for the removal of water, is preferred to spray drying since it requires less energy. More importantly, milk powder produced from evaporated milk has a longer shelf life and larger powder particles with a smaller amount of included air. Jiang and Guo (2014) explain that even though milk is commonly dried by roller drying or spray drying in a stream of hot air, spray drying is more commonly used for infant formula because roller dried products have a lower solubility in water, are susceptible to irreversible component changes during drying, and because roller dried powder has a lower microbiological quality than spray dried powder.

Even though the overall manufacturing process of IF is basically the same, differences in temperatures and other processing parameters can still be observed. A patent filed in 1987 by Angel Gil and Luis Valverde, revealed the exact process used in the manufacturing of IF. The process start mixing together vegetable oil and aqueous products while heating to homogenize and emulsify the contents. Then the addition of nucleotides and other ingredients followed by pasteurized takes place at 95–100°C. Next step uses vacuum to condense the mixture, with a slowly dry at a low temperature, then sterilized it twice at 121°C or once at 151°C for a few seconds (Gil & Valverde, 1985). Another patent also states that infant formula is mixed and homogenized using high pressure and it is then spray dried at temperatures around 200°C (Van Den Brenk, Van Dijke, Van Der Steen, Moonen, & Van Baalen, 2015). Moreover, Van Dijke, et al.'s patent describes the addition of a second emulsification step, pasteurization or heat treatment of the aqueous phase from either 60 to 100°C, or 70 to 90°C, and more preferably to 85°C, with a holding time of 1 s to 6 min, more preferably 10 s to 6 min, and even more preferably from 30 s to 6 min (Van Dijke, Schröder, Ustunel, Halsema, & Moonen, 2018). Moreover, they recommend that the lipid phase should be liquid at the temperature(s) used during the process. However, if the lipid phase is solid due to its composition it is preferably heated to above the melting temperature of at least one lipid, preferably vegetable lipid, contained in the lipid phase, specifically to a temperature above its melting point from 55 to 60°C. It is important to note that besides the variation of parameters involved in the manufacturing process previously mentioned, special attention should be given to other potential oxidation factors such as the IF packaging, handling, and preparation techniques at home that could promote the formation of POPs.

*Figure 7* *(cont'd)*



**TABLE 4** Phytosterols oxidation products (POPs) profile in infant formula

| Phytosterols oxidation products (POPs) concentrations in infant formula | | | | |
|---|---|---|---|---|
| **Type** | **Processing conditions** | **POPs** | **POPs concentration** | **Reference** |
| Powder | N/A | 7-ketositosterol | 0.7–5.0 μg/g lipid | Zunin et al. (1998) |
| Liquid | • Control<br>• Storage (9 months) | 7α-hydroxycampesterol | • 0.9 ± 0.0 μg/100 g (control)<br>• 1.2 ± 0.2 μg/100 g (after 9 months) | García-Llatas et al. (2008) |
| | | 7β-hydroxycampesterol | • 2.4 ± 0.1 μg/100 g (control)<br>• 2.3 ± 0.9 μg/100 g (after 9 months) | |
| | | α-epoxycampesterol | • 1.3 ± 0.3 μg/100 g (control)<br>• 0.8 ± 0.4 μg/100 g (after 9 months) | |
| | | 7-ketocampesterol | • 2.0 ± 0.6 μg/100 g (control)<br>• 1.9 ± 0.5 μg/100 g (after 9 months) | |
| | | 7α-hydroxystigmasterol | • 2.4 ± 0.01 μg/100 g (control)<br>• 1.2 ± 0.2 μg/100 g s (after 9 months) | |
| | | 7β-hydroxystigmasterol | • 2.0 ± 0.2 μg/100 g (control)<br>• 1.1 ± 0.6 μg/100 g (after 9 months) | |
| | | α-epoxystigmasterol | • 3.7 ± 0.4 μg/100 g (control)<br>• 3.3 ± 1.2 μg/100 g (after 9 months) | |
| | | β-epoxystigmasterol | • 1.9 ± 0.1 μg/100 g se (control)<br>• 1.5 ± 0.6 μg/100 g sample (after 9 months) | |
| | | 7-ketostigmasterol | • 1.2 ± 0.4 μg/100 g sample (control)<br>• 1.2 ± 0.1 μg/100 g sample (after 9 months) | |
| | | 7α-hydroxysitosterol | • 1.4 ± 0.1 μg/100 g sample (control)<br>• 1.0 ± 0.4 μg/100 g sample (after 9 months) | |
| | | 7β-hydroxysitosterol | • 5.1 ± 0.8 μg/100 g sample (control)<br>• 4.5 ± 1.3 μg/100 g sample (after 9 months) | |
| | | α-epoxysitosterol | • 12.7 ± 2.2 μg/100 g sample (control)<br>• 10.3 ± 3.5 μg/100 g sample (after 9 months) | |
| | | β-epoxysitosterol | • 5.6 ± 0.9 μg/100 g sample (control)<br>• 4.9 ± 1.5 μg/100 g sample (after 9 months) | |
| | | 7-ketositosterol | • 16.1 ± 1.8 μg/100 g sample (control)<br>• 13.8 ± 2.9 μg/100 g sample (after 9 months) | |

## 8 | THE CHALLENGE OF FOOD PROCESSING: LIPID OXIDATION

Westerns diet contains levels of 150–400 mg/day of PS, being mainly β-sitosterols, campesterol, stigamasterol and traces of saturated phytostanols (Ryan et al., 2009). Therefore, POPs intake should be monitored to ensure low accumulation of these molecules in the human body, especially in infants, since like all unsaturated lipids, PS oxidation can occur during the processing, preparation, and storage of IF (O'Callaghan et al., 2014). During a study of sterol oxidation in IF, it was found that the phytosterols were more oxidized than their animal counterpart, cholesterol (García-Llatas & Rodríguez-Estrada, 2011; González-Larena et al., 2011) pointing out the need of surveillance for these types of food. On the other hand, several studies have addressed the reduction of POPs with addition of antioxidants such as tocopherol, butylated hydroxytoluene (BHT), and plant ethanolic extracts (Rudzińska et al., 2005); however, no efforts have been dedicated to evaluate the different processing technologies and their effects in promoting PS oxidation in IF. It is imperative to build a strong scientific information base regarding not only PS consumption and its oxidized derivatives, but also their toxicity and exposure on a vulnerable population like infants.

Lipid oxidation is a major concern, but other nutritional components can also undergo reactions during food processing. The Maillard reaction is a well-known reaction that occurs in food during thermal processing. A reducing sugar and amino acid react together to form various Maillard reaction products (MRPs), depending on the conditions. The Maillard reactions leads to decreased nutritional value of proteins and some products may have adverse health effects. Milk and milk derivative products, like infant formula, are high in protein and sugar and so thermal processing of milk will lead to the formation of MRPs (Tamanna & Mahmood, 2015). Proteins can also undergo oxidation if exposed to heat, light, or metal. These oxidation reactions can lead to racemization of amino acids. This can lead to major changes in the protein's properties, activity, and structure which could also cause increased toxicity (Chen et al., 2019). Lipid oxidation products can also react further with amino acids (Hematyar, Rustad, Sampels, & Kastrup Dalsgaard, 2019). The processing of infant

*Figure 7* *(cont'd)*



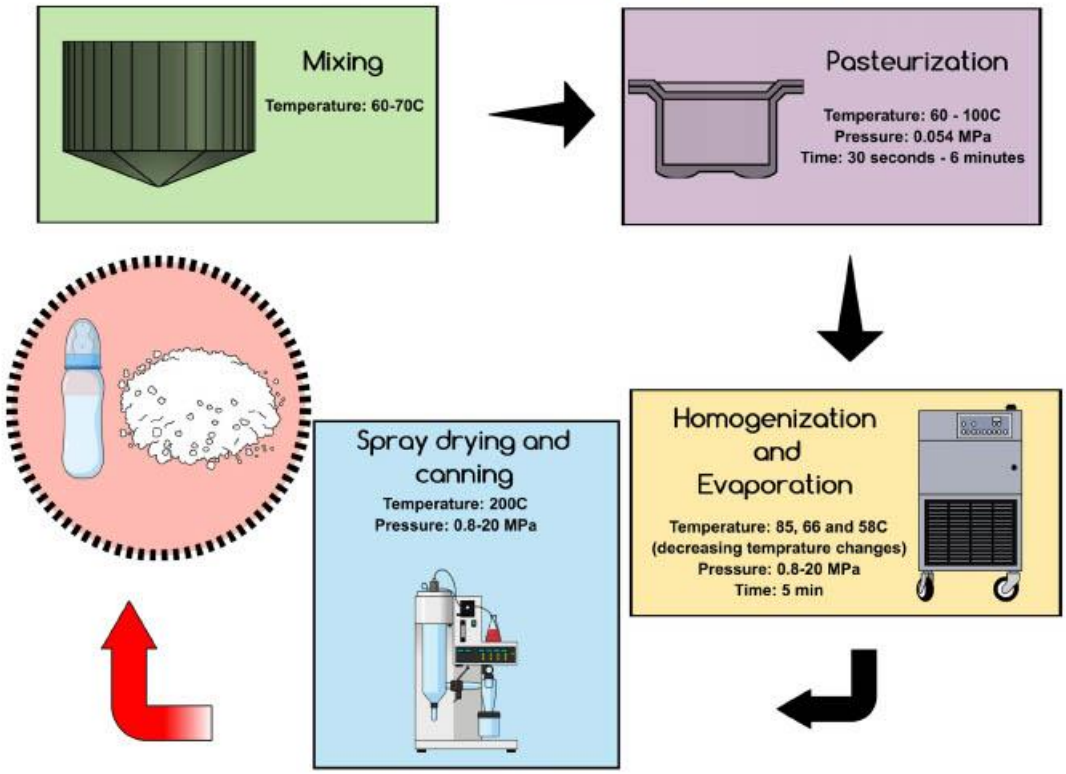


**FIGURE 3** Infant formula milk powder manufacturing process

formula can have drastic effects on the composition of the ingredients inside. High heat leads to lipid and protein oxidation, as well as the Maillard reaction. These reactions can lead to the formation of a wide range of components that can further react and may have drastic effects on infants.

## 9 | CONCLUSION

PS are plant sterols present in vegetable food sources. Their oxidation is promoted by temperature, storage, and processing, generating oxidized derivatives know as POPs. IF is given to infants at a very early age, especially those who have special dietary restriction or medical conditions and cannot fulfill their nutritional needs from breast milk. These young children rely on the contents of infant formula to obtain the nutrition needed for a healthy and complete physical development and growth. However, the scarce information related with POPs, the mechanisms these compounds undergo, and the potential adverse health effect on infants that could result from their intake through infant formula consumption is still unclear and should be of great concern to the scientific community. What is the outcome when the only meal these young children have is filled with oxidized sterols that could affect them for the rest of their life? Even though there are not enough studies of POPs formation due to food processing, there is no doubt that the processing of IF leads to the formation of oxidized sterol products. Therefore, high amounts of phytosterols in infant formula could result in high concentrations of POPs. The effects of infant formula processing in the POPs formation mechanism and their connection with infant's health are still widely unknown. However, it is well known that they can have consequences on human health based on studies performed in different food matrices. A deeper analysis of the processing and treatment of infant formula is crucial to obtain a better understanding of these compounds' formation pathways and their biological mechanism in infants, which are a critical and vulnerable population.

## ACKNOWLEDGMENTS

This study has been partially funded by SHORA Spectrum Health through the grant RC108332. Cristobal Torres-Palacios was supported by Consejo Nacional de Ciencia y Tecnología Fellowship 711082. Authors thank Dr. Carlo Barnaba (MSU) for his critical review of the manuscript.

*Figure 7* (cont'd)



**ORCID**

*Ilce Medina-Meza* https://orcid.org/0000-0001-7712-4018

**REFERENCES**

Adcox, C., Boyd, L., Oehrl, L., Allen, J., & Fenner, G. (2001). Comparative effects of phytosterol oxides and cholesterol oxides in cultured macrophage-derived cell lines. *Journal of Agricultural and Food Chemistry*, *49*(4), 2090–2095.

Alemany, L., Barbera, R., Alegría, A., & Laparra, J. (2014). Plant sterols from foods in inflammation and risk of cardiovascular disease: A real threat? *Food and Chemical Toxicology*, *69*, 140–149.

Awad, A. B., Tagle Hernandez, A. Y., Fink, C. S., & Mendel, S. L. (1997). Effect of dietary phytosterols on cell proliferation and protein kinase C activity in rat colonic mucosa.

Bang, H.-j., Arakawa, C., Takada, M., Sato, M., & Imaizumi, K. (2008). A comparison of the potential unfavorable effects of oxycholesterol and oxyphytosterol in mice: Different effects, on cerebral 24S-hydroxychoelsterol and serum triacylglycerols levels. *Bioscience, Biotechnology, and Biochemistry*, *72*(12), 3128–3133.

Barriuso, B., Otaegui-Arrazola, A., Menéndez-Carreño, M., Astiasarán, I., & Ansorena, D. (2012). Sterols heating: Degradation and formation of their ring-structure polar oxidation products. *Food Chemistry*, *135*(2), 706–712.

Berger, A., Jones, P. J., & Abumweis, S. S. (2004). Plant sterols: Factors affecting their efficacy and safety as functional food ingredients. *Lipids in Health and Disease*, *3*(1), 5.

Boatright, W. L., & Crum, A. D. (2016). Redox cycling and generation of reactive oxygen species in commercial infant formulas. *Food Chemistry*, *196*, 189–195.

Borges Botelho, P., Galasso, M., Dias, V., Mandrioli, M., Pereira Lobato, L., Rodriguez-Estrada, M. T., & Alves Castro, I. (2014). Oxidative stability of functional phytosterol-enriched dark chocolate. *LWT - Food Science and Technology*, *55*(2), 444–451. https://doi.org/10.1016/j.lwt.2013.09.002

Cercaci, L., Rodriguez-Estrada, M. T., Lercker, G., & Decker, E. A. (2007). Phytosterol oxidation in oil-in-water emulsions and bulk oil. *Food Chemistry*, *102*(1), 161–167.

Chen, Z., Leinisch, F., Greco, I., Zhang, W., Shu, N., Chuang, C. Y., … Davies, M. J. (2019). Characterisation and quantification of protein oxidative modifications and amino acid racemisation in powdered infant milk formula. *Free Radical Research*, *53*(1), 68–81.

Comunian, T. A., & Favaro-Trindade, C. S. (2016). Microencapsulation using biopolymers as an alternative to produce food enhanced with phytosterols and omega-3 fatty acids: A review. *Food Hydrocolloids*, *61*, 442–457.

Danesi, F., Ferioli, F., Caboni, M. F., Boschetti, E., Di Nunzio, M., Verardo, V., … Bordoni, A. (2011). Phytosterol supplementation reduces metabolic activity and slows cell growth in cultured rat cardiomyocytes. *British Journal of Nutrition*, *106*(4), 540–548.

Derewiaka, D., & Obiedziński, M. (2012). Phytosterol oxides content in selected thermally processed products. *European Food Research and Technology*, *234*(4), 703–712. https://doi.org/10.1007/s00217-012-1681-3

Descalzo, A. M., Rizzo, S. A., Servent, A., Rossetti, L., Lebrun, M., Pérez, C. D., … Dhuique-Mayer, C. (2018). Oxidative status of a yogurt-like fermented maize product containing phytosterols. *Journal of Food Science and Technology*, *55*(5), 1859–1869. https://doi.org/10.1007/s13197-018-3102-5

Desic, S. D., & Birlouez-Aragon, I. (2011). The FAST index–a highly sensitive indicator of the heat impact on infant formula model. *Food Chemistry*, *124*(3), 1043–1049.

Dutta, P. C. (1997). Studies on phytosterol oxides. II: Content in some vegetable oils and in french fries prepared in these oils. *Journal of the American Oil Chemists Society*, *74*(6), 659–666. https://doi.org/10.1007/s11746-997-0198-6

Dutta, P. C. (1999). Phytosterol oxides in some samples of pure phytosterols mixture and in a few tablet supplement preparations in Finland. *Natural Antioxidants and Anticarcinogens in Nutrition, Health and Disease*, (240), 316–319. https://doi.org/10.1533/9781845698409.5.316

Dutta, P. C., & Appelqvist, L. A. (1997). Studies on phytosterol oxides. I: Effect of storage on the content in potato chips prepared in different vegetable oils. *Journal of the American Oil Chemists Society*, *74*(6), 647–657. https://doi.org/10.1007/s11746-997-0197-7

Expert Panel on Detection, E. (2001). Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *Jama*, *285*(19), 2486.

Ferioli, F., Armaforte, E., & Caboni, M. F. (2014). Comparison of the Lipid Content, Fatty Acid Profile and Sterol Composition in Local Italian and Commercial Royal Jelly Samples. *Journal of the American Oil Chemists' Society*, *91*(6), 875–884. https://doi.org/10.1007/s11746-014-2446-x

Gabay, C., Lamacchia, C., & Palmer, G. (2010). IL-1 pathways in inflammation and human diseases. *Nature Reviews Rheumatology*, *6*(4), 232–241.

García-Llatas, G., Cercaci, L., Rodriguez-Estrada, M. T., Lagarda, M. J., Farré, R., & Lercker, G. (2008). Sterol oxidation in ready-to-eat infant foods during storage. *Journal of Agricultural and Food Chemistry*, *56*(2), 469–475.

García-Llatas, G., & Rodríguez-Estrada, M. T. (2011). Current and new insights on phytosterol oxides in plant sterol-enriched food. *Chemistry and Physics of Lipids*, *164*(6), 607–624.

Gil, A., & Valverde, L. (1985). *Nucleotide enriched humanized milk and process for its preparation*. Google Patents.

Golley, R. K., & Hendrie, G. A. (2014). Evaluation of the relative concentration of serum fatty acids C14: 0, C15: 0 and C17: 0 as markers of children's dairy fat intake. *Annals of Nutrition and Metabolism*, *65*(4), 310–316.

González-Larena, M., García-Llatas, G., Vidal, M. C., Sánchez-Siles, L. M., Barberá, R., & Lagarda, M. J. (2011). Stability of plant sterols in ingredients used in functional foods. *Journal of Agricultural and Food Chemistry*, *59*(8), 3624–3631.

Grandgirard, A., Demaison-Meloche, J., Cordelet, C., & Demaison, L. (2004). Incorporation of oxyphytosterols in tissues of hamster. *Reproduction Nutrition Development*, *44*(6), 599–608.

Grandgirard, A., Martine, L., Demaison, L., Cordelet, C., Joffre, C., Berdeaux, O., & Semon, E. (2004). Oxyphytosterols are present in plasma of healthy human subjects. *British Journal of Nutrition*, *91*(1), 101–106.

Grandgirard, A., Sergiel, J. P., Nour, M., Demaison-Meloche, J., & Gihiès, C. (1999). Lymphatic absorption of phytosterol oxides in rats. *Lipids*, *34*(6), 563–570.

Green Corkins, K., & Shurley, T. (2016). What's in the bottle? A review of infant formulas. *Nutrition in Clinical Practice*, *31*(6), 723–729.

Hageman, J. H., Danielsen, M., Nieuwenhuizen, A. G., Feitsma, A. L., & Dalsgaard, T. K. (2019). Comparison of bovine milk fat and vegetable fat for infant formula: Implications for infant health. *International Dairy Journal*, *92*, 37–49.

Hamdan, I. J., Claumarchirant, L., Garcia-Llatas, G., Alegría, A., & Lagarda, M. J. (2017). Sterols in infant formulas: Validation of a gas chromatographic method. *International Journal of Food Sciences and Nutrition*, *68*(6), 695–703.

Hamdan, I. J., Sanchez-Siles, L. M., Garcia-Llatas, G., & Lagarda, M. a. J. s. (2018). Sterols in infant formulas: A bioaccessibility study. *Journal of Agricultural and Food Chemistry*, *66*(6), 1377–1385.

*Figure 7* *(cont'd)*



Hematyar, N., Rustad, T., Sampels, S., & Kastrup Dalsgaard, T. (2019). Relationship between lipid and protein oxidation in fish. *Aquaculture Research*, *50*(5), 1393–1403.

Hu, H., Liu, H., Shi, A., Liu, L., Fauconnier, M. L., & Wang, Q. (2018). The effect of microwave pretreatment on micronutrient contents, oxidative stability and flavor quality of peanut oil. *Molecules*, *24*(1). https://doi.org/10.3390/molecules24010062

Hu, Y., Wang, M., Huang, W., Yang, G., Lou, T., Lai, S., … Zheng, L. (2018). Risk assessment of dietary exposure to phytosterol oxidation products from baked food in China. *Food Additives & Contaminants: Part A*, *35*(2), 200–210.

Islam, M. A., Jeong, B.-G., Jung, J., Shin, E.-C., Choi, S.-G., & Chun, J. (2017). Phytosterol determination and method validation for selected nuts and seeds. *Food Analytical Methods*, *10*(10), 3225–3234. https://doi.org/10.1007/s12161-017-0877-3

Jiang, Y., & Guo, M. (2014). Processing technology for infant formula. In *Human milk biochemistry and infant formula manufacturing technology* (pp. 211–229). Cambridge, England: Elsevier.

John, R. P., Anisha, G. S., Nampoothiri, K. M., & Pandey, A. (2011). Micro and macroalgal biomass: A renewable source for bioethanol. *Bioresource Technology*, *102*(1), 186–193. https://doi.org/10.1016/j.biortech.2010.06.139

Johnson, D. R., & Decker, E. A. (2015). The role of oxygen in lipid oxidation reactions: A review. *Annual Review of Food Science and Technology*, *6*, 171–190.

Johnsson, L., & Dutta, P. C. (2006a). Determination of phytosterol oxides in some food products by using an optimized transesterification method. *Food Chemistry*, *97*(4), 606–613. https://doi.org/10.1016/j.foodchem.2005.05.037

Johnsson, L., & Dutta, P. C. (2006b). Determination of phytosterol oxides in some food products by using an optimized transesterification method. *Food Chemistry*, *97*(4), 606–613. https://doi.org/10.1016/j.foodchem.2005.05.037

Lagarda, M., García-Llatas, G., & Farré, R. (2006). Analysis of phytosterols in foods. *Journal of Pharmaceutical and Biomedical Analysis*, *41*(5), 1486–1496.

Law, M. (2000). Plant sterol and stanol margarines and health. *BMJ*, *320*(7238), 861–864.

Lea, L., Hepburn, P., Wolfreys, A., & Baldrick, P. (2004). Safety evaluation of phytosterol esters. Part 8. Lack of genotoxicity and subchronic toxicity with phytosterol oxides. *Food and Chemical Toxicology*, *42*(5), 771–783.

Lebovics, V. K., Neszlenyi, K., Latif, S., Somogyi, L., Peredi, J., Farkas, J., & Gaal, O. (1999). Formation of sterol oxides in edible oils. *Natural Antioxidants and Anticarcinogens in Nutrition, Health and Disease*, *240*, 320–322. https://doi.org/10.1533/9781845698409.5.320

Lemaire, M., Le Huërou-Luron, I., & Blat, S. (2018). Effects of infant formula composition on long-term metabolic health. *Journal of Developmental Origins of Health and Disease*, *9*(6), 573–589.

Liang, Y. T., Wong, W. T., Guan, L., Tian, X. Y., Ma, K. Y., Huang, Y., & Chen, Z.-Y. (2011). Effect of phytosterols and their oxidation products on lipoprotein profiles and vascular function in hamster fed a high cholesterol diet. *Atherosclerosis*, *219*(1), 124–133.

Lin, Y., Knol, D., Menéndez-Carreño, M., Blom, W. A. M., Matthee, J., Janssen, H. G., & Trautwein, E. A. (2016). Formation of plant sterol oxidation products in foods during baking and cooking using margarine without and with added plant sterol esters. *Journal of Agricultural and Food Chemistry*, *64*(3), 653–662. https://doi.org/10.1021/acs.jafc.5b04952

Lin, Y., Knol, D., Menéndez-Carreño, M., Baris, R., Janssen, H.-G., & Trautwein, E. A. (2018). Oxidation of sitosterol and campesterol in foods upon cooking with liquid margarines without and with added plant sterol esters. *Food Chemistry*, *241*, 387–396.

Lin, Y., Knol, D., Valk, I., van Andel, V., Friedrichs, S., Lütjohann, D., … Trautwein, E. A. (2017). Thermal stability of plant sterols and formation of their oxidation products in vegetable oils and margarines upon controlled heating. *Chemistry and Physics of Lipids*, *207*, 99–107.

Llaverias, G., Escolà-Gil, J., Lerma, E., Julve, J., Pons, C., Cabré, A., … Blanco-Vaca, F. (2013). Phytosterols inhibit the tumor growth and lipoprotein oxidizability induced by a high-fat diet in mice with inherited breast cancer. *Journal of Nutritional Biochemistry*, *24*(1), 39–48.

Maguire, L., Konoplyannikov, M., Ford, A., Maguire, A. R., & O'brien, N. M. (2003). Comparison of the cytotoxic effects of β-sitosterol oxides and a cholesterol oxide, 7β-hydroxycholesterol, in cultured mammalian cells. *British Journal of Nutrition*, *90*(4), 767–775.

Maldonado, J., Gil, A., Narbona, E., & Molina, J. A. (1998). Special formulas in infant nutrition: A review. *Early Human Development*, *53*, S23–S32.

Maldonado-Pereira, L., Schweiss, M., Barnaba, C., & Medina-Meza, I. G. (2018). The role of cholesterol oxidation products in food toxicity. *Food and Chemical Toxicology*, *118*, 908–939. https://doi.org/10.1016/j.fct.2018.05.059

Mba, O. I., Dumont, M. J., & Ngadi, M. (2015). Palm oil: Processing, characterization and utilization in the food industry – A review. *Food Bioscience*, *10*, 26–41. https://doi.org/10.1016/j.fbio.2015.01.003

McClements, D., Decker, E., & Weiss, J. (2007). Emulsion-based delivery systems for lipophilic bioactive components. *Journal of Food Science*, *72*(8), R109–R124.

Medina-Meza, I. G., & Barnaba, C. (2013). Kinetics of cholesterol oxidation in model systems and foods: Current status. *Food Engineering Reviews*, *5*(3), 171–184.

Medina-Meza, I. G., Barnaba, C., & Barbosa-Cánovas, G. V. (2014). Effects of high pressure processing on lipid oxidation: A review. *Innovative Food Science & Emerging Technologies*, *22*, 1–10.

Mellies, M., Glueck, C., Sweeney, C., Fallat, R., Tsang, R., & Ishikawa, T. (1976). Plasma and dietary phytosterols in children. *Pediatrics*, *57*(1), 60–67.

Menéndez-Carreño, M., Knol, D., & Janssen, H. G. (2016). Development and validation of methodologies for the quantification of phytosterols and phytosterol oxidation products in cooked and baked food products. *Journal of Chromatography A*, *1428*, 316–325. https://doi.org/10.1016/j.chroma.2015.09.073

Meynier, A., Andre, A., Lherminier, J., Grandgirard, A., & Demaison, L. (2005). Dietary oxysterols induce in vivo toxicity of coronary endothelial and smooth muscle cells. *European Journal of Nutrition*, *44*(7), 393–405.

Min, J. S., Khan, M. I., Lee, S. O., Yim, D. G., Seol, K. H., Lee, M., & Jo, C. (2016). Impact of cooking, storage, and reheating conditions on the formation of cholesterol oxidation products in pork loin. *Korean Journal for Food Science of Animal Resources*, *36*(1), 23–28. https://doi.org/10.5851/kosfa.2016.36.1.23

Moreau, R. A., Whitaker, B. D., & Hicks, K. B. (2002). Phytosterols, phytostanols, and their conjugates in foods: Structural diversity, quantitative analysis, and health-promoting uses. *Progress in Lipid Research*, *41*(6), 457–500.

Nestel, P., Cehun, M., Pomeroy, S., Abbey, M., & Weldon, G. (2001). Cholesterol-lowering effects of plant sterol esters and non-esterified stanols in margarine, butter and low-fat foods. *European Journal of Clinical Nutrition*, *55*(12), 1084–1090.

Nguyen, T. T. (1999). The cholesterol-lowering action of plant stanol esters. *The Journal of Nutrition*, *129*(12), 2109–2112.

Nieminen, V., Laakso, P., Kuusisto, P., Niemelä, J., & Laitinen, K. (2016). Plant stanol content remains stable during storage of cholesterol-lowering functional foods. *Food Chemistry*, *196*, 1325–1330. https://doi.org/10.1016/j.foodchem.2015.10.059

Normén, L., Bryngelsson, S., Johnsson, M., Evheden, P., Ellegard, L., Brants, H., … Dutta, P. (2002). The phytosterol content of some cereal foods commonly consumed in Sweden and in the Netherlands. *Journal of Food Composition and Analysis*, *15*(6), 693–704. https://doi.org/10.1006/jfca.2002.1098

*Figure 7* *(cont'd)*



Normén, L., Ellegård, L., Brants, H., Dutta, P., & Andersson, H. (2007). A phytosterol database: Fatty foods consumed in Sweden and The Netherlands. *Journal of Food Composition and Analysis*, *20*(3–4), 193–201.

O'Callaghan, Y., McCarthy, F. O., & O'Brien, N. M. (2014). Recent advances in phytosterol oxidation products. *Biochemical and Biophysical Research Communications*, *446*(3), 786–791.

Ostlund, R. E., Jr. (2002). Phytosterols in human nutrition. *Annual Review of Nutrition*, *22*(1), 533–549.

Ostlund, R. E., Jr., Racette, S. B., Okeke, A., & Stenson, W. F. (2002). Phytosterols that are naturally present in commercial corn oil significantly reduce cholesterol absorption in humans. *The American Journal of Clinical Nutrition*, *75*(6), 1000–1004.

Ostlund, R. E., Jr., Racette, S. B., & Stenson, W. F. (2003). Inhibition of cholesterol absorption by phytosterol-replete wheat germ compared with phytosterol-depleted wheat germ. *The American Journal of Clinical Nutrition*, *77*(6), 1385–1389.

Piironen, V., Toivo, J., & Lampi, A.-M. (2000). Natural sources of dietary plant sterols. *Journal of Food Composition and Analysis*, *13*(4), 619–624.

Pinedo, S., Vissers, M. N., von Bergmann, K., Elharchaoui, K., Lütjohann, D., Luben, R., … Boekholdt, S. M. (2007). Plasma levels of plant sterols and the risk of coronary artery disease: The prospective EPIC-Norfolk population study. *Journal of Lipid Research*, *48*(1), 139–144.

Plumb, J. A., Rhodes, M. J. C., Lampi, A. M., Buchgraber, M., & Kroon, P. A. (2011). Phytosterols in plant foods: Exploring contents, data distribution and aggregated values using an online bioactives database. *Journal of Food Composition and Analysis*, *24*(7), 1024–1031. https://doi.org/10.1016/j.jfca.2010.10.007

Rajasekaran, A., & Kalaivani, M. (2013). Designer foods and their benefits: A review. *Journal of Food Science and Technology*, *50*(1), 1–16.

Riciputi, Y., Serrazaneti, D. I., Verardo, V., Vannini, L., Caboni, M. F., & Lanciotti, R. (2016). Effect of fermentation on the content of bioactive compounds in tofu-type products. *Journal of Functional Foods*, *27*, 131–139. https://doi.org/10.1016/j.jff.2016.08.041

Rossen, L. M., Simon, A. E., & Herrick, K. A. (2016). Types of infant formulas consumed in the United States. *Clinical Pediatrics*, *55*(3), 278–285.

Rudzińska, M., Uchman, W., & Wąsowicz, E. (2005). Plant sterols in food technology. *Acta Scientiarum Polonorum Technologia Alimentaria*, *4*(1), 147–156.

Rudzińska, M., Przybylski, R., & Wąsowicz, E. (2014). Degradation of phytosterols during storage of enriched margarines. *Food Chemistry*, *142*, 294–298. https://doi.org/10.1016/j.foodchem.2013.07.041

Ryan, E., McCarthy, F. O., Maguire, A. R., & O'Brien, N. M. (2009). Phytosterol oxidation products: Their formation, occurrence, and biological effects. *Food Reviews International*, *25*(2), 157–174.

Ryan, E., Galvin, K., O'Connor, T. P., Maguire, A. R., & O'Brien, N. M. (2007). Phytosterol, squalene, tocopherol content and fatty acid profile of selected seeds, grains, and legumes. *Plant Foods for Human Nutrition*, *62*(3), 85–91.

Schött, H.-F., Baumgartner, S., Husche, C., Luister, A., Friedrichs, S., Miller, C. M., … Weingärtner, O. (2017). Oxidation of sitosterol and transport of its 7-oxygenated products from different tissues in humans and ApoE knockout mice. *The Journal of Steroid Biochemistry and Molecular Biology*, *169*, 145–151.

Schött, H.-F., Husche, C., Friedrichs, S., Miller, C. M., McCarthy, F. O., Laufs, U., … Lütjohann, D. (2015). 7β-Hydroxysitosterol crosses the blood–brain barrier more favored than its substrate sitosterol in ApoE −/− mice. *Steroids*, *99*, 178–182.

Scholz, B., Menzel, N., Lander, V., & Engel, K.-H. (2016). Heating two types of enriched margarine: Complementary analysis of phytosteryl/phytostanyl fatty acid esters and phytosterol/phytostanol oxidation products. *Journal of Agricultural and Food Chemistry*, *64*(13), 2699–2708. https://doi.org/10.1021/acs.jafc.6b00617

Shahzad, N., Khan, W., Shadab, M., Ali, A., Saluja, S. S., Sharma, S., … Abdel-Wahab, A. F. (2017). Phytosterols as a natural anticancer agent: Current status and future perspective. *Biomedicine & Pharmacotherapy*, *88*, 786–794.

Srigley, C. T., & Haile, E. A. (2015). Quantification of plant sterols/stanols in foods and dietary supplements containing added phytosterols. *Journal of Food Composition and Analysis*, *40*, 163–176. https://doi.org/10.1016/j.jfca.2015.01.008

Su, M.-Y., Broadhurst, M., Liu, C.-P., Gathercole, J., Cheng, W.-L., Qi, X.-Y., … Haigh, B. (2017). Comparative analysis of human milk and infant formula derived peptides following in vitro digestion. *Food Chemistry*, *221*, 1895–1903.

Tamanna, N., & Mahmood, N. (2015). Food processing and maillard reaction products: Effect on human health and nutrition. *International Journal of Food Science*, *2015*, 1–6.

Tomoyori, H., Kawata, Y., Higuchi, T., Ichi, I., Sato, H., Sato, M., … Imaizumi, K. (2004). Phytosterol oxidation products are absorbed in the intestinal lymphatics in rats but do not accelerate atherosclerosis in apolipoprotein E–deficient mice. *The Journal of Nutrition*, *134*(7), 1690–1696.

Traves, D. (2015). Understanding infant formula. *Paediatrics and Child Health*, *25*(9), 413–417.

Uauy, R., & Dangour, A. D. (2009). Fat and fatty acid requirements and recommendations for infants of 0-2 years and children of 2-18 years. *Annals of Nutrition & Metabolism*, *55*(1–3), 76–96.

Van Den Brenk, J., Van Dijke, K. C., Van Der Steen, A. M. L., Moonen, R. C. J., & Van Baalen, A. (2015). *Process for preparing infant formula*. Google Patents.

Van Dijke, K. C., Schröder, J., Ustunel, M. A., Halsema, D. G. R., & Moonen, R. C. J. (2018). *Two-step emulsification process for preparing infant formula*. Google Patents.

Vandenplas, Y., Zakharova, I., & Dmitrieva, Y. (2015). Oligosaccharides in infant formula: More evidence to validate the role of prebiotics. *British Journal of Nutrition*, *113*(9), 1339–1344.

Verardo, V., Garcia-Salas, P., Baldi, E., Segura-Carretero, A., Fernandez-Gutierrez, A., & Fiorenza Caboni, M. (2014). Pomegranate seeds as a source of nutraceutical oil naturally rich in bioactive lipids. *Food Research International*, *65*, 445–452. https://doi.org/10.1016/j.foodres.2014.04.044

Verhé, R., Verleyen, T., Van Hoed, V., & De Greyt, W. (2006). Influence of refining of vegetable oils on minor components. *Journal of Oil Palm Research*, *4*, 168–179.

Winkler, J. K., Warner, K., & Glynn, M. T. (2007). Effect of deep-fat frying on phytosterol content in oils with differing fatty acid composition. *Journal of the American Oil Chemists' Society*, *84*(11), 1023–1030. https://doi.org/10.1007/s11746-007-1138-1

Zunin, P., Calcagno, C., & Evangelisti, F. (1998). Sterol oxidation in infant milk formulas and milk cereals. *Journal of Dairy Research*, *65*(4), 591–598.

**How to cite this article:** Kilvington A, Maldonado-Pereira L, Torres-Palacios C, Medina-Meza I. Phytosterols and their oxidative products in infant formula. *J Food Process Eng*. 2019; e13151. https://doi.org/10.1111/jfpe.13151

# APPENDIX C

## LIST OF ALL FOOD MEALS, THEIR TEST CODES, AND FOOD GROUP

***Table 6:*** *Processed and Ultra-processed meals ID used in this study*

| | | Sample ID | *Ultra-processed Foods (UPF)* |
|---|---|---|---|
| *Ready to Eat (RTE)* | Dairy | D1 - RTE | American Cheese – Happy Farms |
| | | D2 - RTE | Cheddar cheese – Happy Farms |
| | | D3 - RTE | Margarine (regular, not low-fat, salted) – Countryside Creamery |
| | | *D4 – RTE | Butter – Praire Farms |
| | | D5 - RTE | Cream (half & half) -Meijer |
| | | D6 - RTE | Swiss cheese - Kroger |
| | | D7 - RTE | Cream cheese – Happy Farms |
| | | D8 - RTE | Ice cream (regular, not low-fat, vanilla) - Purple Cow |
| | | D9 - RTE | Yogurt (low-fat, fruit flavored) - Yoplait |
| | | D10 - RTE | Chocolate milk - Nesquick |
| | | D11-RTE | Infant formula – Little Journey |
| | Meat & Poultry | MP1 - RTE | Bologna - Eckrich |
| | | MP2 - RTE | Salami – Oscar Mayer |
| | | MP3 - RTE | Soup bean w/bacon/pork (canned, prepared w/water) - Campbell's |
| | | MP4 – RTE | Chili con carne w/beans (canned) - Campbell's |
| | | MP5 – RTE | Lasagna w/meat (frozen, heated) – Michael Angelo's |
| | | MP6 – RTE | Chicken noodle soup - Kroger |
| | | MP7 – RTE | Beef and vegetables soup – Kroger |
| | | MP8 – RTE | Mini Ravioli - Chef Boyardee |
| | | MP9 - RTE | Spaghetti - Chef Boyardee |
| | Seafood | S1 - RTE | Clam chowder (New England, canned, prep w/ whole milk) – Kroger |
| | Eggs & egg derivatives | E1 - RTE | Mayonnaise (regular, bottled) – Hellmann's |
| | | E2 - RTE | Macaroni salad (from grocery/deli) - Meijer |
| | Baby food | BF1 - RTE | Baby food - beef and broth/gravy – Beech Nut |
| | | BF2 - RTE | Baby food – chicken and broth/gravy - Gerber |
| | | BF3 - RTE | Baby food - vegetables and beef - Gerber |
| | | BF4 - RTE | Baby food - vegetables and chicken - Gerber |
| | | BF5 - RTE | Baby food - chicken noodle dinner - Gerber |
| | | BF6 - RTE | Baby food - macaroni, tomato and cheese - Gerber |
| | | BF7 - RTE | Baby food - turkey and rice - Gerber |
| | | BF8 - RTE | Baby food – turkey and broth/gravy – Beech Nut |
| | | BF9 - RTE | Baby food – fruit yogurt - Gerber |
| | | BF10 - RTE | Baby food – chicken with rice - Gerber |

***Table 6*** *(cont'd)*

| | | | |
|---|---|---|---|
| | | BF11 - RTE | Baby food – vegetables and turkey - Gerber |
| | | BF12 - RTE | Baby food – macaroni and cheese with vegetables - Gerber |
| | | BF13 - RTE | Pasta pick-ups (cheese ravioli) - Gerber |
| | Others | 01 - RTE | Popcorn w/butter (microwave) - Kroger |
| | | 02 - RTE | Salad dressing (creamy/buttermilk type, regular) – Aldi's Tuscan Garden |
| | | 03 - RTE | Macaroni & cheese (boiled) - Kraft |
| | | 04 - RTE | Macaroni & cheese (microwaved) - Kraft |
| | | | |
| *Fast Food (FF* | Meat & Poultry | MP10 - FF | Hamburger on bun – McDonald's |
| | | MP11 - FF | Chicken nuggets – McDonald's |
| | | MP12 - FF | Cheeseburger on bun – McDonald's |
| | | MP13 - FF | Steak tacos w/beans, lettuce, rice and cheese - Chipotle |
| | | MP14 - FF | Cheese and chicken quesadilla - Chipotle |
| | | MP15 - FF | Chicken burrito w/lettuce, cheese, pico - Chipotle |
| | | MP16 - FF | Chicken drumstick - KCF |
| | | MP17 - FF | Chicken wing - KFC |
| | | MP18 - FF | Beef w/vegetables - Panda Express |
| | | MP19 - FF | Chicken w/vegetables - Panda Express |
| | | MP20 - FF | Chicken filet - (broiled sandwich) – Chick Fil'A |
| | | MP21 - FF | Roast beef, ham & Provolone - Jimmy Johns |
| | | MP22 - FF | Sliced turkey and bacon - Jimmy Johns |
| | | MP23 - FF | Supreme pizza - Marco's Pizza |
| | | MP24 - FF | Pepperoni pizza, hand tossed - Domino's |
| | Seafood | S2 - FF | Fish sandwich on bun – McDonald's |
| | | S3 - FF | Fried Shrimp – Panda Express |
| | Others | 05 - FF | French-Fries – McDonald's |
| | | 06 - FF | McDonald's Biscuit - Big Breakfast |
| | | 07 - FF | McDonald's Hotcakes - Big Breakfast |
| | | 08 - FF | Biscuit - KFC |
| | | 09 - FF | French Fries - KFC |
| | | 010 - FF | Mashed potato - KFC |

*The only processed food included in this study.

# APPENDIX D

# CONDITIONS OF THE COLLECTION AND PREPARATION PROCESS OF READY-TO-EAT (RTE) ITEMS

***Table 7:*** *Detailed information for the collection and preparation process of RTE items in this analysis.*

| Sample | Sample Collection | Cooking Conditions | Lipid Extraction |
|---|---|---|---|
| *American cheese – Happy Farms* | Local Grocery Store | NR | Entire Product Used |
| *Cheddar cheese – Happy Farms* | | NR | |
| *Bologna – Eckrich* | | NR | |
| *Salami – Oscar Mayer* | | NR | |
| *Chili con carne w/beans (canned) - Campbell's* | | Microwave: Covered, on high heat for 2 ½ to 3 min. | |
| *Swiss cheese – Kroger* | | NR | |
| *Cream cheese – Happy Farms* | | NR | |
| *Butter – Praire Farms* | | NR | |
| *Margarine (regular, not low-fat, salted) – Countryside Creamery* | | NR | |
| *Mayonnaise (regular, bottled) – Hellmann's* | | NR | |
| *Cream (half & half) - Meijer* | | NR | |
| *Soup bean w/bacon/pork (canned, prepared w/water) - Campbell's* | | Microwave: Covered on high heat for 2 ½ to 3 min. | |
| *Clam chowder (New England, canned, prep w/ whole milk) – Kroger* | | Microwave: High heat for 2 min and then for 30 s at a time until cooked, stirring each time, about 3 min. | |
| *Ice cream (regular, not low-fat, vanilla) - Purple Cow* | | NR | |
| *Macaroni salad (from grocery/deli) - Meijer* | | NR | |
| *Lasagna w/meat (frozen, heated) – Michael Angelo's* | | Oven bake: 25 - 35 min at 400°F. | |
| *Popcorn w/butter (microwave) - Kroger* | | Microwave: 2 – 2 ½ min on high heat. | |
| *Ranch salad dressing (creamy/buttermilk type, regular) – Aldi's Tuscan Garden* | | NR | |
| *Macaroni & cheese (boiled) - Kraft* | | Boil pan: Stir for 7 to 8 min in boiling water. | |

*Table 7 (cont'd)*

| Sample | Sample Collection | Cooking Conditions | Lipid Extraction |
|---|---|---|---|
| *Macaroni & cheese (microwaved) – Kraft* | Local Grocery Store | Microwave: Uncovered, on high heat 8 to 10 min or until water is absorbed, stirring every 3 min. | Entire Product Used |
| *Chicken noodle soup- Kroger* | | Microwave: Covered, on high heat for 4 to 5 min or until hot. | |
| *Beef and vegetables soup – Campbell's* | | Microwave: Covered, on high heat for 4 to 5 min or until hot. | |
| *Baby food - beef and broth/gravy – Beech Nut* | | NR | |
| *Baby food – chicken and broth/gravy - Gerber* | | NR | |
| *Baby food - vegetables and beef - Gerber* | | NR | |
| *Baby food - vegetables and chicken - Gerber* | | NR | |
| *Baby food - chicken noodle dinner - Gerber* | | NR | |
| *Baby food - macaroni, tomato, and cheese - Gerber* | | NR | |
| *Baby food - turkey and rice - Gerber* | | NR | |
| *Yogurt (low-fat, fruit flavored) - Yoplait* | | NR | |
| *Baby food – turkey and broth/gravy – Beech Nut* | | NR | |
| *Baby food – fruit yogurt – Gerber* | | NR | |
| *Baby food – chicken with rice - Gerber* | | NR | |
| *Baby food – vegetables and turkey - Gerber* | | NR | |
| *Baby food – macaroni and cheese with vegetables - Gerber* | | NR | |
| *Infant Formula - Little Journey* | | Mix 1 scoop of powder for every 2 ounces of water. | |
| *Chocolate milk – Nesquick** | | NR | |
| *Mini Ravioli - Chef Boyardee* | | Microwave: 1 minute 30 seconds or until warm on high heat. | |
| *Spaghetti & Meatballs - Chef Boyardee* | | Microwave: 1 minute 30 seconds or until warm on high heat. | |
| *Pasta pick-ups (cheese ravioli) - Gerber* | | NR | |

# APPENDIX E

# CONDITIONS OF THE COLLECTION AND PREPARATION PROCESS OF FAST-FOOD (FF) MEALS

***Table 8:*** *Detailed information for the collection and preparation process of RTE items in this analysis.*

| Sample | Sample Collection | Lipid Extraction |
|---|---|---|
| *Quarter-pound hamburger on bun* | McDonald's | Entire product except bun |
| *Chicken nuggets* | | Entire product used |
| *French-Fries* | | Entire product used |
| *Quarter-pound cheeseburger on bun* | | Entire product except bun |
| *Fish sandwich on bun* | | Entire product except bun |
| *Biscuit - McDonald's Big Breakfast* | | Entire product used |
| *Hotcakes - McDonald's Big Breakfast* | | Entire product used |
| *Taco w/beef, beans, lettuce, rice, and cheese* | Chipotle | Entire product except tortilla |
| *Cheese and chicken quesadilla* | | Entire product used |
| *Steak burrito w/lettuce, cheese, pico* | | Entire product except tortilla |
| *Chicken drumstick (original recipe)* | KFC | Entire product except bones |
| *Chicken wing (original recipe)* | | Entire product except bones |
| *Biscuit* | | Entire product used |
| *French Fries* | | Entire product used |
| *Mashed potato* | | Entire product used |
| *Beef w/vegetables* | Panda Express | Entire product used |
| *Chicken w/vegetables* | | Entire product used |
| *Fried Shrimp* | | Entire product used |
| *Chicken filet (broiled sandwich)* | Chick Fil' A | Entire product except bun |
| *Roast beef, ham & Provolone* | Jimmy Johns | Entire product except bread |
| *Sliced turkey and bacon* | | Entire product except bread |
| *Supreme pizza* | Marco's Pizza | Entire product used |
| *Pepperoni and cheese pizza* | Domino's Pizza | Entire product used |

# APPENDIX F

## STEROLS AND PHYTOSTEROLS CONTENT IN READY-TO-EAT (RTE) AND FAST-FOOD (FF) MEALS

***Table 9:*** *Unpublished results of the sterols and phytosterols content in RTE and FF meals in UPFs*

| *Ready to Eat* | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Food Category* | *Food Item* | *Brassicasterol* | *Campesterol* | *Stigmasterol* | *β-Sitosterol* | *Fucosterol* | *β-Tocopherol* |
| | | *(mg/100 g fat) ±STD* | | | | | |
| *Dairy* | D1-RTE | ND | 26.01 ± 9.31 | 20.30 ± 6.01 | 86.64 ± 12.43 | ND | ND |
| | D2-RTE | ND | ND | ND | ND | ND | ND |
| | D3-RTE | ND | 33.31 ± 14.70 | 43.37 ± 12.51 | 136.05 ± 5.72 | ND | ND |
| | D4-RTE | ND | ND | ND | ND | ND | ND |
| | D5-RTE | ND | ND | ND | ND | ND | ND |
| | D6-RTE | ND | ND | ND | ND | ND | ND |
| | D7-RTE | ND | ND | ND | ND | ND | ND |
| | D8-RTE | ND | ND | ND | ND | ND | ND |
| | D9-RTE | ND | ND | ND | ND | ND | ND |
| | D10-RTE | ND | ND | ND | ND | ND | ND |
| | D11-RTE | ND | 18.19 ± 4.86 | 11.64 ± 5.32 | 95.02 ± 24.75 | ND | ND |
| *Meat & Poultry* | MP1-RTE | ND | ND | ND | ND | ND | ND |
| | MP2-RTE | ND | ND | ND | tr | ND | ND |
| | MP3-RTE | ND | 68.45 ± 13.99 | 259.84 ± 40.55 | 626.48 ± 57.01 | 58.18 ± 26.67 | ND |
| | MP4-RTE | ND | ND | 31.84 ± 9.45 | 102.34 ± 29.25 | ND | ND |
| | MP5-RTE | ND | 14.60 ± 5.20 | ND | ND | 0.033 ± 0.026 | ND |
| | MP6-RTE | ND | ND | ND | ND | ND | ND |
| | MP7-RTE | ND | ND | ND | ND | ND | ND |
| | MP8-RTE | ND | 36.47 ± 24.74 | tr | 83.75 ± 43.50 | ND | ND |
| | MP9-RTE | ND | ND | ND | ND | ND | ND |
| *Seafood* | S1-RTE | ND | 33.99 ± 26.75 | tr | 51.87 ± 37.21 | ND | ND |
| *Eggs & egg's derivatives* | E1-RTE | ND | 61.64 ± 24.67 | 42.06 ± 13.92 | 176.90 ± 18.66 | ND | ND |
| | E2-RTE | ND | 53.46 ± 6.89 | 37.17 ± 9.46 | 162.59 ± 24.23 | ND | ND |
| *Baby foods* | BF1-RTE | ND | ND | ND | ND | ND | ND |
| | BF2-RTE | ND | ND | ND | ND | ND | ND |
| | BF3-RTE | ND | 10.48 ± 5.14 | 6.52 ± 1.87 | 47.10 ± 13.21 | ND | ND |
| | BF4-RTE | ND | 182.76 ± 53.20 | 75.16 ± 41.86 | 498.98 ± 129.71 | ND | ND |

*Table 9* *(cont'd)*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **BF5-RTE** | ND | 136.48 ± 47.29 | 33.77 ± 7.19 | 391.77 ± 93.78 | ND | ND |
| | **BF6-RTE** | ND | 21.98 ± 6.84 | ND | 100.31 ± 19.76 | ND | ND |
| | **BF7-RTE** | ND | 190.88 ± 21.15 | ND | 421.59 ± 137.27 | ND | ND |
| | **BF8-RTE** | ND | ND | ND | ND | ND | ND |
| | **BF9-RTE** | ND | ND | ND | ND | ND | ND |
| | **BF10-RTE** | ND | 48.06 ± 9.69 | 25.41 ± 14.33 | 160.14 ± 26.56 | ND | ND |
| | **BF11-RTE** | 19.72 ± 3.21 | ND | 48.31 ± 2.23 | 435.62 ± 41.06 | ND | ND |
| | **BF12-RTE** | 29.60 ± 3.30 | 99.33 ± 24.73 | ND | 236.18 ± 18.19 | ND | ND |
| | **BF13-RTE** | ND | 44.86 ± 3.55 | ND | ND | 0.046 ± 0.014 | ND |
| *Others* | **O1-RTE** | ND | 34.54 ± 5.32 | ND | ND | ND | ND |
| | **O2-RTE** | ND | 50.87 ± 14.28 | 38.09 ± 10.27 | 148.17 ± 8.02 | ND | 0.019 ± 0.012 |
| | **O3-RTE** | ND | 38.65 ± 19.12 | ND | ND | 5.45 ± 1.58 | ND |
| | **O4-RTE** | ND | 26.01 ± 9.31 | 16.34 ± 9.22 | 124.15 ± 12.47 | 39.85 ± 17.26 | ND |
| | | | | *Fast Food* | | | |
| *Food Category* | *Food Item* | *Brassicasterol* | *Campesterol* | *Stigmasterol* | *β-Sitosterol* | *Fucosterol* | *β-Tocopherol* |
| | | | | | *(mg/100 g fat) ±STD* | | |
| *Meat & Poultry* | **MP10-FF** | ND | ND | ND | ND | ND | ND |
| | **MP11-FF** | ND | 122.85 ± 18.26 | ND | 296.67 ± 38.94 | ND | ND |
| | **MP12-FF** | ND | ND | ND | ND | ND | ND |
| | **MP13-FF** | ND | 66.46 ± 14.08 | 26.36 ± 10.01 | 176.24 ± 26.14 | ND | ND |
| | **MP14-FF** | ND | 81.36 ± 13.67 | 209.33 ± 40.88 | ND | ND | ND |
| | **MP15-FF** | ND | ND | 53.03 ± 7.46 | ND | ND | ND |
| | **MP16-FF** | ND | 86.20 ± 33.23 | ND | 165.55 ± 63.66 | ND | ND |
| | **MP17-FF** | 27.56 ± 4.66 | 101.79 ± 8.40 | ND | 173.08 ± 17.49 | ND | ND |
| | **MP18-FF** | ND | 68.98 ± 23.39 | 48.25 ± 13.69 | 343.35 ± 137.26 | ND | ND |
| | **MP19-FF** | ND | 4.77 ± 3.44 | ND | 33.40 ± 17.52 | ND | ND |
| | **MP20-FF** | ND | 22.44 ± 7.49 | 8.93 ± 7.17 | 95.24 ± 9.40 | ND | ND |
| | **MP21-FF** | ND | ND | ND | 39.39 ± 6.79 | ND | ND |

*Table 9 (cont'd)*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | MP22-FF | ND | 20.69 ± 10.72 | ND | 34.28 ± 4.46 | ND | ND |
| | MP23-FF | ND | 83.95 ± 7.74 | 168.10 ± 24.33 | ND | ND | ND |
| | MP24-FF | ND | **23.57 ± 7.87** | ND | 68.94 ± 6.79 | ND | ND |
| *Seafood* | S2-FF | ND | 60.10 ± 22.86 | ND | 199.39 ± 26.35 | ND | ND |
| | S3-FF | ND | 25.42 ± 4.88 | 19.73 ± 13.05 | 36.94 ± 4.58 | ND | ND |
| *Others* | 05-FF | ND | 192.00 ± 21.55 | ND | 331.87 ± 34.11 | ND | ND |
| | 06-FF | ND | ND | 36.45 ± 17.21 | ND | ND | ND |
| | 07-FF | ND | 40.86 ± 5.16 | 224.87 ± 30.79 | ND | ND | ND |
| | 08-FF | ND | 35.93 ± 23.68 | ND | 74.36 ± 8.20 | ND | ND |
| | 09-FF | 44.19 ± 17.67 | 184.08 ± 25.17 | ND | 333.41 ± 45.98 | ND | ND |
| | 010-FF | ND | 60.98 ± 12.72 | ND | 102.64 ± 19.30 | tr | ND |

# LITERATURE CITED

# LITERATURE CITED


(EPA), E. P. A. (2018). What We Eat in America Food Commodity Intake Database (2005-2010). Retrieved from http://fcid.foodrisk.org/

Adamczyk, M., Scherrer, E., Kupferberg, A., Malviya, A. N., & Mersel, M. (1998). Inhibition of p42/p44 mitogen-activated protein kinase by oxysterols in rat astrocyte primary cultures and C6 glioma cell lines. Journal of Neuroscience Research, 53(1), 38-50. doi:https://doi.org/10.1002/(SICI)1097-4547(19980701)53:1<38::AID-JNR5>3.0.CO;2-K

Adcox, C., Boyd, L., Oehrl, L., Allen, J., & Fenner, G. (2001). Comparative effects of phytosterol oxides and cholesterol oxides in cultured macrophage-derived cell lines. J Agric Food Chem, 49(4), 2090-2095. doi:10.1021/jf001175v

Al-Ismail, K. (2002). Effect of two methods of grilling on the oxidative rancidity and cholesterol oxidation in beef and chicken shawerma. Grasas Y Aceites, 53(3), 335-339.

Al-Ismail, K., Herzallah, S., & Humied, M. (2007). Effect of processing and storage of Jameed on conjugated linoleic acid content and fat and cholesterol oxidation. LWT-Food Science and Technology, 40(3), 454-459.

Aldini, G., Yeum, K.-J., Niki, E., & Russell, R. M. (2010). Biomarkers for antioxidant defense and oxidative damage : principles and practical applications (K.-J. Y. Giancarlo Aldini, Estuo Niki, and Robert M. Russell Ed. First Edition ed.): Wiley.

Alemany, L., Barbera, R., Alegría, A., & Laparra, J. M. (2014). Plant sterols from foods in inflammation and risk of cardiovascular disease: a real threat? Food Chem Toxicol, 69, 140-149. doi:10.1016/j.fct.2014.03.038

Alkazemi, D., Egeland, G., Vaya, J., Meltzer, S., & Kubow, S. (2008). Oxysterol as a marker of atherogenic dyslipidemia in adolescence. J Clin Endocrinol Metab, 93(11), 4282-4289. doi:10.1210/jc.2008-0586

Amrutha Kala, A. L. (2014). Studies on saturated and trans fatty acids composition of few commercial brands of biscuits sold in Indian market. J Food Sci Technol, 51(11), 3520-3526. doi:10.1007/s13197-014-1421-8

Andrea Serra and Giuseppe Conte and Alice Cappucci and Laura Casarosa and Marcello, M. Cholesterol and Fatty Acids Oxidation in Meat from Three Muscles of Massese Suckling Lambs Slaughtered at Different Weights. Italian Journal of Animal Science, 13(3), 3275. doi:10.4081/ijas.2014.3275

Angulo, A. J., Romera, J. M., Ramirez, M., & Gil, A. (1997). Determination of cholesterol oxides in dairy products. Effect of storage conditions. Journal of Agricultural and Food Chemistry, 45(11), 4318-4323. doi:10.1021/jf970431+

Avsaroglu, M. D., Buzrul, S., Alpas, H., Akcelik, M., & Bozoglu, F. (2006). Use of the Weibull model for lactococcal bacterlophage inactivation by high hydrostatic pressure. International Journal of Food Microbiology, 108(1), 78-83. doi:10.1016/j.ijfoodmicro.2005.10.027

Awad, A. B., Hernandez, A. Y., Fink, C. S., & Mendel, S. L. (1997). Effect of dietary phytosterols on cell proliferation and protein kinase C activity in rat colonic mucosa. Nutr Cancer, 27(2), 210-215. doi:10.1080/01635589709514527

Awogbemi, O., Onuh, E. I., & Inambao, F. L. (2019). Comparative study of properties and fatty acid composition of some neat vegetable oils and waste cooking oils. International Journal of Low-Carbon Technologies, 14(3), 417-425. doi:10.1093/ijlct/ctz038

Aziz, N. H., Mahrous, S. R., & Youssef, B. M. (2002). Effect of gamma-ray and microwave treatment on the shelf-life of beef products stored at 5degreeC. Food Control, 13(6-7), 437-444.

Baggio, S., & Bragagnolo, N. (2006). Cholesterol oxide, cholesterol, total lipid and fatty acid contents in processed meat products during storage. LWT-Food Science and Technology, 39(5), 513-520.

Bang, H. J., Arakawa, C., Takada, M., Sato, M., & Imaizumi, K. (2008). A Comparison of the Potential Unfavorable Effects of Oxycholesterol and Oxyphytosterol in Mice: Different Effects, on Cerebral 24S-Hydroxychoelsterol and Serum Triacylglycerols Levels. Bioscience Biotechnology and Biochemistry, 72(12), 3128-3133. doi:10.1271/bbb.80256

Barnaba, C., Rodriguez-Estrada, M. T., Lercker, G., Sergio Garcia, H., & Medina-Meza, I. G. (2016). Cholesterol photo-oxidation: A chemical reaction network for kinetic modeling. Steroids, 116, 52-59. doi:10.1016/j.steroids.2016.10.004

Barriuso, B., Ansorena, D., Poyato, C., & Astiasarán, I. (2015). Cholesterol and stigmasterol within a sunflower oil matrix: Thermal degradation and oxysterols formation. Steroids, 99, 155-160. doi:https://doi.org/10.1016/j.steroids.2015.02.009

Barriuso, B., Mariutti, L., Ansorena, D., Astiasarán, I., & Bragagnolo, N. (2016). Solanum sessiliflorum (mana-cubiu) antioxidant protective effect toward cholesterol oxidation: Influence of docosahexaenoic acid. European Journal of Lipid Science and Technology, 118(8), 1125-1131. doi:https://doi.org/10.1002/ejlt.201500285

Barriuso, B., Otaegui-Arrazola, A., Menéndez-Carreño, M., Astiasarán, I., & Ansorena, D. (2012). Sterols heating: Degradation and formation of their ring-structure polar oxidation products. Food Chemistry, 135(2), 706-712. doi:https://doi.org/10.1016/j.foodchem.2012.05.027

Beltran, E., Pla, R., Capellas, M., Yuste, J., & Mor-Mur, M. (2004). Lipid oxidation and colour in pressure- and heat-treated minced chicken thighs. Journal of the Science of Food and Agriculture, 84(11), 1285-1289. doi:10.1002/jsfa.1778

Bemrah, N., Sirot, V., Leblanc, J. C., & Volatier, J. L. (2009). Fish and seafood consumption and omega 3 intake in French coastal populations: CALIPSO survey. Public Health Nutr, 12(5), 599-608. doi:10.1017/S1368980008002681

Berger, A., Jones, P. J., & Abumweis, S. S. (2004). Plant sterols: factors affecting their efficacy and safety as functional food ingredients. Lipids Health Dis, 3, 5. doi:10.1186/1476-511X-3-5

Berthier, A., Lemaire-Ewing, S., Prunet, C., Montange, T., Vejux, A., Pais de Barros, J. P., . . . Neel, D. (2005). 7-Ketocholesterol-induced apoptosis. Involvement of several pro-apoptotic but also anti-

apoptotic calcium-dependent transduction pathways. FEBS J, 272(12), 3093-3104. doi:10.1111/j.1742-4658.2005.04723.x

Beyza Vurusaner and Gabriella Leonarduzzi and Paola Gamba and Giuseppe Poli and Huveyda, B. (2016). Oxysterols and mechanisms of survival signaling. Molecular Aspects of Medicine, 49, 8-22. doi:https://doi.org/10.1016/j.mam.2016.02.004

Beyza Vurusaner and Paola Gamba and Gabriella Testa and Simona Gargiulo and Gabriella Leonarduzzi and Giuseppe Poli and Huveyda, B. (2016). 82 - Molecular Mechanism of Oxysterol Induced Survival Response: The Role of Autophagy and ROS. Free Radical Biology and Medicine, 100, S46. doi:https://doi.org/10.1016/j.freeradbiomed.2016.10.123

Biasi, F., Chiarpotto, E., Sottero, B., Maina, M., Mascia, C., Guina, T., . . . Poli, G. (2013). Evidence of cell damage induced by major components of a diet-compatible mixture of oxysterols in human colon cancer CaCo-2 cell line. Biochimie, 95(3), 632-640. doi:10.1016/j.biochi.2012.10.011

Biasi, F., Mascia, C., Astegiano, M., Chiarpotto, E., Nano, M., Vizio, B., . . . Poli, G. (2009). Pro-oxidant and proapoptotic effects of cholesterol oxidation products on human colonic epithelial cells: a potential mechanism of inflammatory bowel disease progression. Free Radic Biol Med, 47(12), 1731-1741. doi:10.1016/j.freeradbiomed.2009.09.020

Bilska, A., Rudzińska, M., Kowalski, R., & Krysztofiak, K. (2009). The effect of soy hydrolysates on changes in cholesterol content and its oxidation products in fine-ground model sausages. Acta Scientiarum Polonorum Technologia Alimentaria, 8(3), 15-22.

Bjorkhem, I., Lovgren-Sandblom, A., Leoni, V., Meaney, S., Brodin, L., Salveson, L., . . . Svenningsson, P. (2013). Oxysterols and Parkinson's disease: Evidence that levels of 24S-hydroxycholesterol in cerebrospinal fluid correlates with the duration of the disease. Neuroscience Letters, 555, 102-105. doi:10.1016/j.neulet.2013.09.003

Boatright, & Crum. (2016). Redox cycling and generation of reactive oxygen species in commercial infant formulas. Food Chemistry, 196, 189-195. doi:https://doi.org/10.1016/j.foodchem.2015.08.130

Bobe, G., Hammond, E. G., Freeman, A. E., Lindberg, G. L., & Beitz, D. C. (2003). Texture of butter from cows with different milk fatty acid compositions. J Dairy Sci, 86(10), 3122-3127. doi:10.3168/jds.S0022-0302(03)73913-7

Bordoni, A., & Capozzi, F. (2015). The foodomics approach for discovering biomarkers of food consumption in nutrition studies. Current Opinion in Food Science, 4, 124-128. doi:https://doi.org/10.1016/j.cofs.2015.07.005

Bosco, D. A., Fowler, D. M., Zhang, Q., Nieva, J., Powers, E. T., Wentworth, P., . . . Kelly, J. W. (2006). Elevated levels of oxidized cholesterol metabolites in Lewy body disease brains accelerate alpha-synuclein fibrilization. Nat Chem Biol, 2(5), 249-253. doi:10.1038/nchembio782

Boselli, E., Velazco, V., Caboni, M. F., & Lercker, G. (2001). Pressurized liquid extraction of lipids for the determination of oxysterols in egg-containing food. J Chromatogr A, 917(1-2), 239-244.

Bretillon, L., Diczfalusy, U., Björkhem, I., Maire, M. A., Martine, L., Joffre, C., . . . Creuzot-Garcher, C. (2007). Cholesterol-24S-hydroxylase (CYP46A1) Is Specifically Expressed in Neurons of the Neural Retina. Current Eye Research, 32(4), 361-366. doi:10.1080/02713680701231857

Britt, C. a. G. E. A. a. G. J. I. a. B. A. M. (1998). Influence of Cherry Tissue on Lipid Oxidation and Heterocyclic Aromatic Amine Formation in Ground Beef Patties. Journal of Agricultural and Food Chemistry, 46(12), 4891-4897. doi:10.1021/jf980233y

Broncano, J. M., Petron, M. J., Parra, V., & Timon, M. L. (2009). Effect of different cooking methods on lipid oxidation and formation of free cholesterol oxidation products (COPs) in Latissimus dorsi muscle of Iberian pigs. Meat Sci, 83(3), 431-437. doi:10.1016/j.meatsci.2009.06.021

Brzeska, M., Szymczyk, K., & Szterk, A. (2016). Current Knowledge about Oxysterols: A Review. Journal of Food Science, 81(10), R2299-R2308. doi:10.1111/1750-3841.13423

Buck, R. J., Ozkaynak, H., Xue, J. P., Zartarian, V. G., & Hammerstrom, K. (2001). Modeled estimates of chlorpyrifos exposure and dose for the Minnesota and Arizona NHEXAS populations. Journal of Exposure Analysis and Environmental Epidemiology, 11(3), 253-268. doi:10.1038/sj.jea.7500164

Calderón-Santiago, M., Peralbo-Molina, Á., Priego-Capote, F., & Luque de Castro María, D. (2012). Cholesterol oxidation products in milk: Processing formation and determination. European Journal of Lipid Science and Technology, 114(6), 687-694. doi:10.1002/ejlt.201100139

Carpenter, K. L., Kirkpatrick, P. J., Weissberg, P. L., Challis, I. R., Dennis, I. F., Freeman, M. A., & Mitchinson, M. J. (2003). Oral alpha-tocopherol supplementation inhibits lipid oxidation in established human atherosclerotic lesions. Free Radic Res, 37(11), 1235-1244.

Cercaci, L., Rodriguez-Estrada, M. T., Lercker, G., & Decker, E. A. (2007). Phytosterol oxidation in oil-in-water emulsions and bulk oil. Food Chemistry, 102(1), 161-167. doi:https://doi.org/10.1016/j.foodchem.2006.05.010

Chen, L. J., Lu, Y. F., Chien, J. T., & Chen, B. H. (2010). Formation and Inhibition of Cholesterol Oxidation Products in Tea-Leaf Eggs during Marinating. Journal of Agricultural and Food Chemistry, 58(19), 10467-10474. doi:10.1021/jf102487j

Chen, Y., T., C. J., Stephen, I. B., & Bing Huei, C. (2012). Formation and Inhibition of Cholesterol Oxidation Products during Marinating of Pig Feet. Journal of Agricultural and Food Chemistry, 60(1), 173-179. doi:10.1021/jf204158a

Chen, Y. S., Aluwi, N. A., Saunders, S. R., Ganjyal, G. M., & Medina-Meza, I. G. (2019). Metabolic fingerprinting unveils quinoa oil as a source of bioactive phytochemicals. Food Chem, 286, 592-599. doi:10.1016/j.foodchem.2019.02.016

Chien, J. T., Wang, H. C., & Chen, B. H. (1998). Kinetic Model of the Cholesterol Oxidation during Heating. Journal of Agricultural and Food Chemistry, 46(7), 2572-2577. doi:10.1021/jf970788d

Chiu, C.-W. a. K. T.-H. a. C. B.-H. (2018). Improved Analytical Method for Determination of Cholesterol-Oxidation Products in Meat and Animal Fat by QuEChERS Coupled with Gas Chromatography–Mass Spectrometry. Journal of Agricultural and Food Chemistry, 66(13), 3561-3571. doi:10.1021/acs.jafc.8b00250

Colles, S. M., Maxson, J. M., Carlson, S. G., & Chisolm, G. M. (2001). Oxidized LDL-induced injury and apoptosis in atherosclerosis. Potential roles for oxysterols. Trends Cardiovasc Med, 11(3-4), 131-138.

Comunian, T. A., & Favaro-Trindade, C. S. (2016). Microencapsulation using biopolymers as an alternative to produce food enhanced with phytosterols and omega-3 fatty acids: A review. Food Hydrocolloids, 61, 442-457. doi:https://doi.org/10.1016/j.foodhyd.2016.06.003

Cote, I., Andersen, M. E., Ankley, G. T., Barone, S., Birnbaum, L. S., Boekelheide, K., . . . DeWoskin, R. S. (2016). The Next Generation of Risk Assessment Multi-Year Study—Highlights of Findings, Applications to Risk Assessment, and Future Directions. Environmental Health Perspectives, 124(11), 1671-1682. doi:doi:10.1289/EHP233

Cruz, P., Torres, C., Ramírez, M. E., Epuñán, M. J., Valladares, L. E., & Sierralta, W. D. (2010). Proliferation of human mammary cancer cells exposed to 27-hydroxycholesterol. Exp Ther Med, 1(3), 531-536. doi:10.3892/etm_00000084

Czech, A., Grela, E. R., & Ognik, K. (2015). Effect of Frying on Nutrients Content and Fatty Acid Composition of Muscles of Selected Freezing Seafoods. Journal of Food and Nutrition Research, 3(1), 9-14.

Damjanovic-Desic, S., & Birlouez-Aragon , I. (2011). The FAST index – A highly sensitive indicator of the heat impact on infant formula model. Food Chemistry, 124(3), 1043-1049. doi:https://doi.org/10.1016/j.foodchem.2010.07.071

Damjanovic-Desic, S., & Birlouez-Aragon, I. (2011). The FAST index – A highly sensitive indicator of the heat impact on infant formula model. Food Chemistry, 124(3), 1043-1049. doi:https://doi.org/10.1016/j.foodchem.2010.07.071

Danesi, F., Ferioli , F., Fiorenza Caboni , M., Boschetti , E., Di Nunzio , M., Verardo , V., . . . Bordoni, A. (2011). Phytosterol supplementation reduces metabolic activity and slows cell growth in cultured rat cardiomyocytes. Br J Nutr. doi:10.1017/S0007114511000626.

Del Rio, D., Rodriguez-Mateo, A., Spencer, J., Tognolini, M., Borges, G., & Crozier, A. (2013). Dietary (Poly)phenolics in Human Health: Structures, Bioavailability, and Evidence of Protective Effects Against Chronic Diseases. Antioxidants & Redox Signaling, 18(14), 1818-1892. doi:10.1089/ars.2012.4581

Derewiaka, D., & Molinska nee Sosinska, E. (2015). Cholesterol transformations during heat treatment. Food Chem, 171, 233-240. doi:10.1016/j.foodchem.2014.08.117

Derewiaka, D., & Obiedzinski, M. (2010). Cholesterol oxides content in selected animal products determined by GC-MS. European Journal of Lipid Science and Technology, 112(10), 1130-1137. doi:10.1002/ejlt.200900238

Derewiaka, D., & Obiedziński, M. (2009). Oxysterol content in selected meats and meat products. Acta Scientiarum Polonorum Technologia Alimentaria, 8(3), 5-13.

Dionisi, F., Golay, P., Aeschlimann, J., & Fay, L. (1998). Determination of cholesterol oxidation products in milk powders: methods comparison and validation. Journal of agricultural and food chemistry, 46(6), 2227-2233.

Dorne, J., Bordajandi, L. R., Amzal, B., Ferrari, P., & Verger, P. (2009). Combining analytical techniques, exposure assessment and biological effects for risk assessment of chemicals in food. Trac-Trends in Analytical Chemistry, 28(6), 695-707. doi:10.1016/j.trac.2009.03.008

Du, M., & Ahn, D. U. (2000). Effects of antioxidants and packaging on lipid and cholesterol oxidation and color changes of irradiated egg yolk powder. Journal of Food Science, 65(4), 625-629. doi:10.1111/j.1365-2621.2000.tb16062.x

Echarte, M., Conchillo, A., Ansorena, D., & Astiasarán, I. (2004). Evaluation of the nutritional aspects and cholesterol oxidation products of pork liver and fish patés. Food Chemistry, 86(1), 47 - 53. doi:https://doi.org/10.1016/j.foodchem.2003.08.027

Egeghy, P. P., Sheldon, L. S., Isaacs, K. K., Özkaynak, H., Goldsmith, M.-R., Wambaugh, J. F., . . . Buckley, T. J. (2016). Computational exposure science: An emerging discipline to support 21st-century risk assessment. Environmental health perspectives, 124(6), 697.

Ekiz, E., & Oz, F. (2019). The effects of different frying oils on the formation of heterocyclic aromatic amines in meatballs and the changes in fatty acid compositions of meatballs and frying oils. Journal of the Science of Food and Agriculture, 99(4), 1509-1518. doi:https://doi.org/10.1002/jsfa.9325

Emanuel, H. A., Hassel, C. A., Addis, P. B., Bergmann, S. D., & Zavoral, J. H. (1991). Plasma Cholesterol Oxidation Products (Oxysterols) in Human Subjects Fed a Meal Rich in Oxysterols. Journal of Food Science, 56(3), 843-847. doi:https://doi.org/10.1111/j.1365-2621.1991.tb05396.x

FDA. (2014). Total dietary study. Food Science Research.

FDA. (2016). Labeling for Infant Formula: Guidance for Industry. Retrieved from https://www.fda.gov/media/99701/download

FDA. (2018). Total Dietary Study. Retrieved from https://www.fda.gov/food/science-research-food/total-diet-study

FDA. (2020). Tittle 21, Volume 2, Chapter 1, Sub chapter B- Food for human consumption. Retrieved 01/05/2021 https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=117.3

Folch, J., Lees, M., & Sloane Stanley, G. (1957). A simple method for the isolation and purification of total lipides from animal tissues. J biol Chem, 226(1), 497-509.

FoodSafety.gov. (2020a). 4 Steps to Food Safety. Retrieved from https://www.foodsafety.gov/keep-food-safe/4-steps-to-food-safety

FoodSafety.gov. (2020b). Meat and Poultry Roasting Charts. Retrieved from FoodSafety.gov

Fukuchi, J., Hiipakka, R. A., Kokontis, J. M., Hsu, S., Ko, A. L., Fitzgerald, M. L., & Liao, S. (2004). Androgenic suppression of ATP-binding cassette transporter A1 expression in LNCaP human prostate cancer cells. Cancer Res, 64(21), 7682-7685. doi:10.1158/0008-5472.CAN-04-2647

Gabay, C., Lamacchia, C., & Palmer, G. (2010). IL-1 pathways in inflammation and human diseases. Nature Reviews Rheumatology, 6(4), 232-241. doi:10.1038/nrrheum.2010.4

Garcia-Cruset, S., Carpenter, K. L. H., Guardiola, F., Stein, B. K., & Mitchinson, M. J. (2001). Oxysterol profiles of normal human arteries, fatty streaks and advanced lesions. Free radical research, 35(1), 31-41.

García-Llatas, G., Cercaci, L., Rodriguez-Estrada, M. T., Lagarda, M. J., Farré, R., & Lercker, G. (2008). Sterol oxidation in ready-to-eat infant foods during storage. J Agric Food Chem, 56(2), 469-475. doi:10.1021/jf072475g

García-Llatas, G., & Rodríguez-Estrada, M. T. (2011). Current and new insights on phytosterol oxides in plant sterol-enriched food. Chem Phys Lipids, 164(6), 607-624. doi:10.1016/j.chemphyslip.2011.06.005

Gargiulo, S., Gamba, P., Testa, G., Leonarduzzi, G., & Poli, G. (2016). The role of oxysterols in vascular ageing. Journal of Physiology-London, 594(8), 2095-2113. doi:10.1113/jp271168

Gargiulo, S., Gamba, P., Testa, G., Rossin, D., Biasi, F., Poli, G., & Leonarduzzi, G. (2015). Relation between TLR4/NF-κB signaling pathway activation by 27-hydroxycholesterol and 4-hydroxynonenal, and atherosclerotic plaque instability. Aging Cell, 14(4), 569-581. doi:10.1111/acel.12322

Gil, A., & Valverde, L. (1982).

Girão, H., Mota, M. C., Ramalho, J., & Pereira, P. (1998). Cholesterol oxides accumulate in human cataracts. Exp Eye Res, 66(5), 645-652. doi:10.1006/exer.1998.0465

Golley, R. K., & Hendrie, G. A. (2014). Evaluation of the Relative Concentration of Serum Fatty Acids C14:0, C15:0 and C17:0 as Markers of Children's Dairy Fat Intake. Annals of Nutrition and Metabolism, 65(4), 310-316. doi:10.1159/000368325

González-Larena, M., García-Llatas, G., Vidal, M. C., Sánchez-Siles, L. M., Barberá, R., & Lagarda, M. J. (2011). Stability of plant sterols in ingredients used in functional foods. J Agric Food Chem, 59(8), 3624-3631. doi:10.1021/jf1044102

Gonçalves Albuquerque, T., Santos, J., Silva, M., Oliveira, M., & Costa, H. (2018). An update on processed foods: Relationship between salt, saturated and trans fatty acids contents. Food Chemistry, 267. doi:10.1016/j.foodchem.2018.01.029

Gorassini, A., Verardo, G., Fregolent, S.-C., & Bortolomeazzi, R. (2017). Rapid determination of cholesterol oxidation products in milk powder based products by reversed phase SPE and HPLC-APCI-MS/MS. Food Chemistry, 230, 604 - 610. doi:https://doi.org/10.1016/j.foodchem.2017.03.080

Grandgirard, A., Demaison-Meloche, J., Cordelet, C., & Demaison, L. (2004). Incorporation of oxyphytosterols in tissues of hamster. Reprod Nutr Dev, 44(6), 599-608. doi:10.1051/rnd:2004058

Grandgirard, A., Guardiola, F., Dutta, P., Codony, R., & Savage, G. (2002). Biological effects of phytosterol oxidation products, future research areas and concluding remarks. Cholesterol and phytosterol oxidation products: analysis, occurrence, and biological effects, 375-382.

Grandgirard, A., Martine, L., Demaison, L., Cordelet, C., Joffre, C., Berdeaux, O., & Semon, E. (2004). Oxyphytosterols are present in plasma of healthy human subjects. Br J Nutr, 91(1), 101-106. doi:10.1079/bjn20031025

Grandgirard, A., Sergiel, J. P., Nour, M., Demaison-Meloche, J., & Giniès, C. (1999). Lymphatic absorption of phytosterol oxides in rats. Lipids, 34(6), 563-570.

Grau, A., Codony, R., Grimpa, S., Baucells, M. D., & Guardiola, F. (2001). Cholesterol oxidation in frozen dark chicken meat: influence of dietary fat source, and alpha-tocopherol and ascorbic acid supplementation. Meat Science, 57(2), 197-208. doi:10.1016/s0309-1740(00)00094-2

Green Corkins, K., & Shurley, T. (2016). What's in the Bottle? A Review of Infant Formulas. Nutr Clin Pract, 31(6), 723-729. doi:10.1177/0884533616669362

Griffiths, W., Abdel-Khalik, J., Yutuc, E., Morgan, A., Gilmore, I., Hearn, T., & Wang, Y. (2017). Cholesterolomics: An update. Analytical Biochemistry, 524, 56-67. doi:https://doi.org/10.1016/j.ab.2017.01.009

Gruenke, L. D., Wrensch, M. R., Petrakis, N. L., Miike, R., Ernster, V. L., & Craig, J. C. (1987). Breast fluid cholesterol and cholesterol epoxides: relationship to breast cancer risk factors and other characteristics. Cancer Res, 47(20), 5483-5487.

Gérard Lizard and Carole Miguet and Ginette Besséde and Serge Monier and Serge Gueldry and Dominique Neel and Philippe, G. (2000). Impairment with various antioxidants of the loss of mitochondrial transmembrane potential and of the cytosolic release of cytochrome c occuring during 7-ketocholesterol-induced apoptosis. Free Radical Biology and Medicine, 28(5), 743-753. doi:https://doi.org/10.1016/S0891-5849(00)00163-5

Haak, L., De Smet, S., Fremaut, D., Walleghem, K., & Raes, K. (2008). Fatty acid profile and oxidative stability of pork influence by duration and time of dietary linseed or fish oil supplementation. Journal of animal science, 86, 1418-1425. doi:10.2527/jas.2007-0032

Hageman, J., Danielsen, M., Nieuwenhuizen, A. G., Feitsma, A. L., & Dalsgaard, T. K. (2019). Comparison of bovine milk fat and vegetable fat for infant formula: Implications for infant health. International Dairy Journal, 92, 37-49. doi:https://doi.org/10.1016/j.idairyj.2019.01.005

Hamdan, I. J. A., Claumarchirant, L., Garcia-Llatas, G., Alegría, A., & Lagarda, M. J. (2017). Sterols in infant formulas: validation of a gas chromatographic method. Int J Food Sci Nutr, 68(6), 695-703. doi:10.1080/09637486.2017.1287883

Hamdan, I. J. A., Sanchez-Siles, L. M., Garcia-Llatas, G., & Lagarda, M. J. (2018). Sterols in Infant Formulas: A Bioaccessibility Study. J Agric Food Chem, 66(6), 1377-1385. doi:10.1021/acs.jafc.7b04635

Hanlon, P., Brorby, G. P., & Krishan, M. (2016). A Risk-Based Strategy for Evaluating Mitigation Options for Process-Formed Compounds in Food Workshop Proceedings. International journal of toxicology, 1091581816640262.

Helmschrodt, C., Becker, S., Schroter, J., Hecht, M., Aust, G., & Thiery, J. (2013). Fast LC–MS/MS analysis of free oxysterols derived from reactive oxygen species in human plasma and carotid plaque. Clinica chimica acta, 425, 3-8.

Hematyar, N., Rustad, T., Sampels, S., & Kastrup Dalsgaard, T. (2019). Relationship between lipid and protein oxidation in fish. Aquaculture Research, 50(5), 1393-1403. doi:https://doi.org/10.1111/are.14012

Hernández, J. a. O. F. A. a. V.-A. G. a. S.-R. I. a. R.-E. M. a. S. G. H. (2014). Cholesterol oxidation and astaxanthin degradation in shrimp during sun drying and storage. 145C, 832-839.

Herzallah, S. M. (2005). Influence of Microwaving and Conventional Heating of Milk on Cholesterol. Pakistan Journal of Nutrition, 4(2), 85-88.

Hintze, K. J., Benninghoff, A. D., & Ward, R. E. (2012). Formulation of the Total Western Diet (TWD) as a Basal Diet for Rodent Cancer Studies. Journal of Agricultural and Food Chemistry, 60(27), 6736-6742. doi:10.1021/jf204509a

Homma, Y., Kondo, Y., Kaneko, M., Kitamura, T., Nyou, W. T., Yanagisawa, M., . . . Kakizoe, T. (2004). Promotion of carcinogenesis and oxidative stress by dietary cholesterol in rat prostate. Carcinogenesis, 25(6), 1011-1014. doi:10.1093/carcin/bgh105

Hore, P., Zartarian, V., Xue, J., Özkaynak, H., Wang, S., Yang, Y., . . . Lioy, P. J. (2006). Children's residential exposure to chlorpyrifos: Application of CPPAES field measurements of chlorpyrifos and TCPy within MENTOR/SHEDS-Pesticides model. Science of The Total Environment, 366(2), 525-537. doi:https://doi.org/10.1016/j.scitotenv.2005.10.012

Hu, F. B. (2002). Dietary pattern analysis: a new direction in nutritional epidemiology. Curr Opin Lipidol, 13(1), 3-9. doi:10.1097/00041433-200202000-00002

Hu, Y., Wang, M., Huang, W., Yang, G., Lou, T., Lai, S., . . . Zheng, L. (2018). Risk assessment of dietary exposure to phytosterol oxidation products from baked food in China. Food Addit Contam Part A Chem Anal Control Expo Risk Assess, 35(2), 200-210. doi:10.1080/19440049.2017.1382727

Huang, J., Wang, Y., Yang, L., Peng, X., Zheng, J., & Ou, S. (2017). Effect of maize bran feruloylated oligosaccharides on the formation of endogenous contaminants and the appearance and textural properties of biscuits. Food Chemistry, 245. doi:10.1016/j.foodchem.2017.11.075

Hur, S. J., Lee, S. Y., & Lee, S. J. (2015). Effect of biopolymer encapsulation on the digestibility of lipid and cholesterol oxidation products in beef during in vitro human digestion. Food Chem, 166, 254-260. doi:10.1016/j.foodchem.2014.06.009

Hur, S. J., Park, G. B., & Joo, S. T. (2007a). A comparison of the effects of dietary conjugated linoleic acid contents, cholesterol, lipid oxidation and drip loss in pork loin and chicken breast. Journal of Muscle Foods, 18(3), 264-275. doi:10.1111/j.1745-4573.2007.00082.x

Hur, S. J., Park, G. B., & Joo, S. T. (2007b). Formation of cholesterol oxidation products (COPs) in animal products. Food Control, 18(8), 939-947. doi:10.1016/j.foodcont.2006.05.008

Ikegami, T., Honda, A., Miyazaki, T., Kohjima, M., Nakamuta, M., & Matsuzaki, Y. (2014). Increased serum oxysterol concentrations in patients with chronic hepatitis C virus infection. Biochem Biophys Res Commun, 446(3), 736-740. doi:10.1016/j.bbrc.2014.01.176

Ilce Gabriela Medina-Meza and Carlo Barnaba and Gustavo, V. B.-C. (2014). Effects of high pressure processing on lipid oxidation: A review. Innovative Food Science & Emerging Technologies, 22, 1-10. doi:https://doi.org/10.1016/j.ifset.2013.10.012

Iuliano, L. (2011). Pathways of cholesterol oxidation via non-enzymatic mechanisms. Chemistry and Physics of Lipids, 164(6), 457-468. doi:10.1016/j.chemphyslip.2011.06.006

Iuliano, L., Micheletta, F., Natoli, S., Ginanni Corradini, S., Iappelli, M., Elisei, W., . . . Diczfalusy, U. (2003). Measurement of oxysterols and alpha-tocopherol in plasma and tissue samples as indices of oxidant stress status. Anal Biochem, 312(2), 217-223.

Jacobsen, C. (2015). Some strategies for the stabilization of long chain n-3 PUFA-enriched foods: A review. European Journal of Lipid Science and Technology, 117(11), 1853-1866. doi:https://doi.org/10.1002/ejlt.201500137

Jain, N., Dürr, U., & Ayyalusamy, R. (2015). Bioanalytical methods for metabolomic profiling: Detection of head and neck cancer, including oral cancer. Chinese Chemical Letters, 26(4), 407-415. doi:https://doi.org/10.1016/j.cclet.2015.03.001

Jasminka Giacometti and Alena Buretić Tomljanović and Djuro, J. (2013). Application of proteomics and metabolomics for investigation of food toxins. Food Research International, 54(1), 1042-1051. doi:https://doi.org/10.1016/j.foodres.2012.10.019

Javitt, N. B., & Javitt, J. C. (2009). The retinal oxysterol pathway: a unifying hypothesis for the cause of age-related macular degeneration. Current Opinion in Ophthalmology, 20(3), 151-157. doi:10.1097/ICU.0b013e32832af468

Jiang, Y. J., & Guo, M. (2014). 8 - Processing technology for infant formula. In M. Guo (Ed.), Human Milk Biochemistry and Infant Formula Manufacturing Technology (pp. 211-229): Woodhead Publishing.

Jo, C., Ahn, D. U., & Lee, J. I. (1999). Lipid and cholesterol oxidation, color changes, and volatile production in irradiated raw pork batters with different fat content. Journal of Food Quality, 22(6), 641-651.

Johnson, D. R., & Decker, E. A. (2015). The Role of Oxygen in Lipid Oxidation Reactions: A Review. Annual Review of Food Science and Technology, 6(1), 171-190. doi:10.1146/annurev-food-022814-015532

Joshi, M., Darshane, P., & Beke, M. (2020). POTENTIAL MEANS TO LOWER CHOLESTEROL OXIDATION PRODUCTS (COPs)

IN THE DIET: A REVIEW. INTERNATIONAL JOURNAL OF CURRENT MEDICAL AND

PHARMACEUTICAL RESEARCH.

Jusakul, A., Yongvanit, P., Loilome, W., Namwat, N., & Kuver, R. (2011). Mechanisms of oxysterol-induced carcinogenesis. Lipids Health Dis, 10, 44. doi:10.1186/1476-511x-10-44

Kang, K. A., Chae, S., Lee, K. H., Park, M. T., Lee, S. J., Lee, Y. S., & Hyun, J. W. (2005). Cytotoxic effect of 7beta-hydroxycholesterol on human NCI-H460 lung cancer cells. Biol Pharm Bull, 28(8), 1377-1380.

Kantor, L. (2016). Americans' Seafood Consumption Below Recommendations. Retrieved from https://www.ers.usda.gov/amber-waves/2016/october/americans-seafood-consumption-below-recommendations/

Karakok, S. G., OzogulL, Y., Saler, M., & Ozogul, F. (2010). PROXIMATE ANALYSIS. FATTY ACID PROFILES AND MINERAL CONTENTS OF MEATS: A COMPARATIVE STUDY. Journal of Muscle Foods, 21(2), 210-223. doi:https://doi.org/10.1111/j.1745-4573.2009.00177.x

Katan, T., Xue, X., Caballero-Solares, A., Taylor, R. G., Rise, M. L., & Parrish, C. C. (2020). Influence of Dietary Long-Chain Polyunsaturated Fatty Acids and ω6 to ω3 Ratios on Head Kidney Lipid Composition and Expression of Fatty Acid and Eicosanoid Metabolism Genes in Atlantic Salmon (Salmo salar). Frontiers in Molecular Biosciences, 7(402). doi:10.3389/fmolb.2020.602587

KFC. (2020). Biscuit. Retrieved from https://www.kfc.com/full-nutrition-guide

Khan, M. I., Min, J. S., Lee, S. O., Yim, D. G., Seol, K. H., Lee, M., & Jo, C. (2015). Cooking, storage, and reheating effect on the formation of cholesterol oxidation products in processed meat products. Lipids Health Dis, 14, 89. doi:10.1186/s12944-015-0091-5

Kilvington, A., Barnaba, C., Rajasekaran, S., Laurens Leimanis, M. L., & Medina-Meza, I. G. (2021). Lipid profiling and dietary assessment of infant formulas reveal high intakes of major cholesterol oxidative product (7-ketocholesterol). Food Chem, 354, 129529. doi:10.1016/j.foodchem.2021.129529

Kilvington, A., Maldonado-Pereira, L., Torres-Palacios, C., & Medina-Meza, I. (2019). Phytosterols and their oxidative products in infant formula. Journal of Food Process Engineering, e13151.

Kotwal, V. (2016). Codex Alimentarius Commission: Role in International Food Standards Setting. 197-205. doi:https://doi.org/10.1016/B978-0-12-384947-2.00397-4

Kreilaus, F., Spiro, A. S., McLean, C. A., Garner, B., & Jenner, A. M. (2016). Evidence for altered cholesterol metabolism in Huntington's disease post mortem brain tissue. Neuropathol Appl Neurobiol, 42(6), 535-546. doi:10.1111/nan.12286

Kulig, W., Cwiklik, L., Jurkiewicz, P., Rog, T., & Vattulainen, I. (2016). Cholesterol oxidation products and their biological importance. Chem Phys Lipids, 199, 144-160. doi:10.1016/j.chemphyslip.2016.03.001

Kumar, N. (1992). Effect of processing conditions on the oxidation of cholesterol in ghee. Journal of the science of food and agriculture, 58(2), 267-273.

Kuver, R. (2012). Mechanisms of oxysterol-induced disease: Insights from the biliary system. Clinical lipidology, 7, 537-548. doi:10.2217/clp.12.53

Lagarda, M., García-Llatas, G., & Farré, R. (2006). Analysis of phytosterols in foods. Journal of Pharmaceutical and Biomedical Analysis, 41(5), 1486 - 1496. doi:https://doi.org/10.1016/j.jpba.2006.02.052

Lappano, R., Recchia, A. G., De Francesco, E. M., Angelone, T., Cerra, M. C., Picard, D., & Maggiolini, M. (2011). The cholesterol metabolite 25-hydroxycholesterol activates estrogen receptor alpha-mediated signaling in cancer cells and in cardiomyocytes. PLoS One, 6(1), e16631. doi:10.1371/journal.pone.0016631

Law, M. (2000). Plant sterol and stanol margarines and health. BMJ, 320(7238), 861-864. doi:10.1136/bmj.320.7238.861

Lea, L. J., Hepburn, P. A., Wolfreys, A. M., & Baldrick, P. (2004). Safety evaluation of phytosterol esters. Part 8. Lack of genotoxicity and subchronic toxicity with phytosterol oxides. Food and Chemical Toxicology, 42(5), 771-783. doi:https://doi.org/10.1016/j.fct.2003.12.014

Ledoux, M., Chardigny, J.-M., Darbois, M., Soustre, Y., Sébédio, J.-L., & Laloux, L. (2005). Fatty acid composition of French butters, with special emphasis on conjugated linoleic acid (CLA) isomers. Journal of Food Composition and Analysis, 18(5), 409-425. doi:https://doi.org/10.1016/j.jfca.2004.01.001

Lee, C. Y., Seet, R. C., Huang, S. H., Long, L. H., & Halliwell, B. (2009). Different patterns of oxidized lipid products in plasma and urine of dengue fever, stroke, and Parkinson's disease patients: cautions in the use of biomarkers of oxidative stress. Antioxid Redox Signal, 11(3), 407-420. doi:10.1089/ars.2008.2179

Lee, T. S., & Chau, L. Y. (2001). Fas/Fas ligand-mediated death pathway is involved in oxLDL-induced apoptosis in vascular smooth muscle cells. American Journal of Physiology-Cell Physiology, 280(3), C709-C718. doi:10.1152/ajpcell.2001.280.3.C709

Lemaire, M., Le Huërou-Luron, I., & Blat, S. (2018). Effects of infant formula composition on long-term metabolic health. J Dev Orig Health Dis, 9(6), 573-589. doi:10.1017/s2040174417000964

Lemaire, S., Lizard, G., Monier, S., Miguet, C., Gueldry, S., Volot, F., . . . Néel, D. (1998). Different patterns of IL-1β secretion, adhesion molecule expression and apoptosis induction in human endothelial cells treated with 7α-, 7β-hydroxycholesterol, or 7-ketocholesterol. FEBS letters, 440(3), 434-439.

Lemaire-Ewing, S., Prunet, C., Montange, T., Vejux, A., Berthier, A., Bessede, G., . . . Lizard, G. (2005). Comparison of the cytotoxic, pro-oxidant and pro-inflammatory characteristics of different oxysterols. Cell Biol Toxicol, 21(2), 97-114. doi:10.1007/s10565-005-0141-2

Leonarduzzi, G., Gargiulo, S., Gamba, P., Perrelli, M. G., Castellano, I., Sapino, A., . . . Poli, G. (2010). Molecular signaling operated by a diet-compatible mixture of oxysterols in up-regulating CD36 receptor in CD68 positive cells. Molecular nutrition & food research, 54 Suppl 1, S31-41. doi:10.1002/mnfr.200900493

Leonarduzzi, G., Poli, G., Sottero, B., & Biasi, F. (2007). Activation of the mitochondrial pathway of apoptosis by oxysterols. Front Biosci, 12, 791-799.

Leonarduzzi, G., Sottero, B., Verde, V., Poli, G., Preedy, V., & Watson, R. (2005). Oxidized products of cholesterol: toxic effect. Reviews in food and nutrition toxicity, 3.

Leoni, V., & Caccia, C. (2011). Oxysterols as biomarkers in neurodegenerative diseases. Chemistry and Physics of Lipids, 164(6), 515-524. doi:10.1016/j.chemphyslip.2011.04.002

Leoni, V., Long, J. D., Mills, J. A., Di Donato, S., Paulsen, J. S., & group, P.-H. s. (2013). Plasma 24S-hydroxycholesterol correlation with markers of Huntington disease progression. Neurobiol Dis, 55, 37-43. doi:10.1016/j.nbd.2013.03.013

Leoni, V., Mariotti, C., Tabrizi, S. J., Valenza, M., Wild, E. J., Henley, S. M., . . . Di Donato, S. (2008). Plasma 24S-hydroxycholesterol and caudate MRI in pre-manifest and early Huntington's disease. Brain, 131(Pt 11), 2851-2859. doi:10.1093/brain/awn212

Let, M. B., Jacobsen, C., & Meyer, A. S. (2007). Lipid Oxidation in Milk, Yoghurt, and Salad Dressing Enriched with Neat Fish Oil or Pre-Emulsified Fish Oil. Journal of Agricultural and Food Chemistry, 55(19), 7802-7809. doi:10.1021/jf070830x

Li, S. C. X., Cherian, G., Ahn, D. U., Hardin, R. T., & Sim, J. S. (1996). Storage, heating, and tocopherols affect cholesterol oxide formation in food oils. Journal of Agricultural and Food Chemistry, 44(12), 3830-3834. doi:10.1021/jf950732o

Liang, Y. T., Wong, W. T., Guan, L., Tian, X. Y., Ma, K. Y., Huang, Y., & Chen, Z. Y. (2011). Effect of phytosterols and their oxidation products on lipoprotein profiles and vascular function in hamster fed a high cholesterol diet. Atherosclerosis, 219(1), 124-133. doi:10.1016/j.atherosclerosis.2011.06.004

Lin, Y., Knol, D., Menéndez-Carreño, M., Baris, R., Janssen, H. G., & Trautwein, E. A. (2018). Oxidation of sitosterol and campesterol in foods upon cooking with liquid margarines without and with added plant sterol esters. Food Chem, 241, 387-396. doi:10.1016/j.foodchem.2017.08.118

Lin, Y., Knol, D., Valk, I., van Andel, V., Friedrichs, S., Lütjohann, D., . . . Trautwein, E. A. (2017). Thermal stability of plant sterols and formation of their oxidation products in vegetable oils and margarines upon controlled heating. Chem Phys Lipids, 207(Pt B), 99-107. doi:10.1016/j.chemphyslip.2017.01.007

Linder, M. E., & Deschenes, R. J. (2007). Palmitoylation: policing protein stability and traffic. Nature Reviews Molecular Cell Biology, 8(1), 74-84. doi:10.1038/nrm2084

Linseisen, J., Wolfram, G., & Miller, A. B. (2002). Plasma 7beta-hydroxycholesterol as a possible predictor of lung cancer risk. Cancer Epidemiol Biomarkers Prev, 11(12), 1630-1637.

Liu, H., Yuan, L., Xu, S., Zhang, T., & Wang, K. (2004). Cholestane-3beta, 5alpha, 6beta-triol promotes vascular smooth muscle cells calcification. Life Sci, 76(5), 533-543. doi:10.1016/j.lfs.2004.06.025

Liu, Y., Mattsson Hultén, L., & Wiklund, O. (1997). Macrophages Isolated From Human Atherosclerotic Plaques Produce IL-8, and Oxysterols May Have a Regulatory Function for IL-8 Production. Arteriosclerosis, Thrombosis, and Vascular Biology, 17(2), 317-323. doi:doi:10.1161/01.ATV.17.2.317

Liu, Z., Rochfort, S., & Cocks, B. G. (2016). Optimization of a single phase method for lipid extraction from milk. J Chromatogr A, 1458, 145-149. doi:10.1016/j.chroma.2016.06.055

Llaverias, Escolà-Gil, C., Lerma, Julve, Pons, Cabré, . . . Blanco-Vaca. (2013). Phytosterols inhibit the tumor growth and lipoprotein oxidizability induced by a high-fat diet in mice with inherited breast cancer. The Journal of Nutritional Biochemistry, 24(1), 39-48. doi:https://doi.org/10.1016/j.jnutbio.2012.01.007

Lopes, T., Cañedo, M., Oliveira, F., & Alcantara, G. (2018). Toward Precision Nutrition: Commercial Infant Formulas and Human Milk Compared for Stereospecific Distribution of Fatty Acids Using Metabolomics. OMICS: A Journal of Integrative Biology, 22(7), 484-492. doi:10.1089/omi.2018.0064

Lopez, J. a. T. S. (2015). Mitochondrial apoptosis: Killing cancer using the enemy within. British Journal of Cancer, 112. doi:10.1038/bjc.2015.85

Lordan, S., Mackrill, J., & O'Brien, N. M. (2009). Oxysterols and mechanisms of apoptotic signaling: implications in the pathology of degenerative diseases. The Journal of Nutritional Biochemistry, 20(5), 321-336. doi:https://doi.org/10.1016/j.jnutbio.2009.01.001

Luu, C. M. a. J. C. D. a. J. B. a. M. O. R. a. P. B. a. H. H. a. B. (1991). Membrane-related oxysterol function: preliminary results on the modification of protein kinase C activity and substrate phosphorylation by 7ß,25-dihydroxycholesterol. Biochimie, 73(10), 1321-1326. doi:https://doi.org/10.1016/0300-9084(91)90096-J

Lütjohann, D., Papassotiropoulos, A., Björkhem, I., Locatelli, S., Bagli, M., Oehring, R. D., . . . Heun, R. (2000). Plasma 24S-hydroxycholesterol (cerebrosterol) is increased in Alzheimer and vascular demented patients. J Lipid Res, 41(2), 195-198.

Maerker, G., & Jones, K. C. (1993a). A-ring oxidation products from γ-irradiation of cholesterol in liposomes. Journal of the American Oil Chemists' Society, 70(3), 255-259. doi:10.1007/bf02545305

Maerker, G., & Jones, K. C. (1993b). A-RING OXIDATION-PRODUCTS FROM GAMMA-IRRADIATION OF CHOLESTEROL IN LIPOSOMES. Journal of the American Oil Chemists Society, 70(3), 255-259. doi:10.1007/bf02545305

Maldonado, J., Gil, A., Narbona, E., & Molina, J. A. (1998). Special formulas in infant nutrition: a review. Early Hum Dev, 53 Suppl, S23-32. doi:10.1016/s0378-3782(98)00062-0

Maldonado-Pereira, L., Schweiss, M., Barnaba, C., & Medina-Meza, I. G. (2018). The role of cholesterol oxidation products in food toxicity. Food and Chemical Toxicology, 118, 908-939. doi:10.1016/j.fct.2018.05.059

Manuelian, C. L., Currò, S., Penasa, M., Cassandro, M., & De Marchi, M. (2017). Prediction of minerals, fatty acid composition and cholesterol content of commercial cheeses by near infrared transmittance spectroscopy. International Dairy Journal, 71, 107-113. doi:https://doi.org/10.1016/j.idairyj.2017.03.011

Mar Roldan and Teresa Antequera and Monica Armenteros and Jorge, R. (2014). Effect of different temperature–time combinations on lipid and protein oxidation of sous-vide cooked lamb loins. Food Chemistry, 149, 129-136. doi:https://doi.org/10.1016/j.foodchem.2013.10.079

Mariutti, L. R. B., & Bragagnolo, N. (2017). Influence of salt on lipid oxidation in meat and seafood products: A review. Food Research International, 94, 90-100. doi:10.1016/j.foodres.2017.02.003

Martin, C. R., Ling, P. R., & Blackburn, G. L. (2016). Review of Infant Feeding: Key Features of Breast Milk and Infant Formula. Nutrients, 8(5). doi:10.3390/nu8050279

Marwarha, G., & Ghribi, O. (2014). Does the oxysterol 27-hydroxycholesterol underlie Alzheimer's disease-Parkinson's disease overlap? Exp Gerontol, 68, 13-18. doi:10.1016/j.exger.2014.09.013

Mazalli, M. R., & Bragagnolo, N. (2007). Effect of storage on cholesterol oxide formation and fatty acid alterations in egg powder. J Agric Food Chem, 55(7), 2743-2748. doi:10.1021/jf063267b

Mazalli, M. R., & Bragagnolo, N. (2009). Increase of Cholesterol Oxidation and Decrease of PUFA as a Result of Thermal Processing and Storage in Eggs Enriched with n-3 Fatty Acids. Journal of Agricultural and Food Chemistry, 57(11), 5028-5034. doi:10.1021/jf901187j

McClements, D. J., Decker, E. A., & Weiss, J. (2007). Emulsion-Based Delivery Systems for Lipophilic Bioactive Components. Journal of Food Science, 72(8), R109-R124. doi:https://doi.org/10.1111/j.1750-3841.2007.00507.x

McCluskey, S. a. D., R. (1993). Validation of chromatographic analysis of cholesterol oxides in dried foods. Trends in Food Science & Technology, 4(6), 175-178. doi:https://doi.org/10.1016/0924-2244(93)90120-Y

McDonald's. (2017). Nutrition Calculator. Retrieved from https://www.mcdonalds.com/us/en-us/about-our-food/nutrition-calculator.html

Medina Meza, I., & Barnaba, C. (2013). Kinetics of Cholesterol Oxidation in Model Systems and Foods: Current Status. Food Engineering Reviews, 5(3), 171-184. doi:10.1007/s12393-013-9069-0

Medina Meza, I. G., Rodriguez-Estrada, M., Lercker, G., Soto-Rodríguez, I., & Garcia, H. (2011). Unusual oxidative pattern in thermo-oxidation of dry films of cholesterol. Revista Mexicana de Ingeniería Química, 10, 47-57.

Medina-Meza, I., & Barnaba, C. (2013). Kinetics of Cholesterol Oxidation in Model Systems and Foods: Current Status. Food Engineering Reviews, 5(3), 171-184. doi:10.1007/s12393-013-9069-0

Medina-Meza, I. G., & Barbosa-Cánovas, G. V. (2015). Assisted extraction of bioactive compounds from plum and grape peels by ultrasonics and pulsed electric fields. Journal of Food Engineering, 166, 268-275.

Medina-Meza, I. G., & Barnaba, C. (2013). Kinetics of cholesterol oxidation in model systems and foods: current status. Food Engineering Reviews, 5(3), 171-184.

Medina-Meza, I. G., Barnaba, C., & Barbosa-Cánovas, G. (2014). Effects of high pressure processing on lipid oxidation: A review. Innovative Food Science & Emerging Technologies, 22, 1-10. doi:https://doi.org/10.1016/j.ifset.2013.10.012

Medina-Meza, I. G., Rodriguez-Estrada, M. T., Sergio Garcia, H., & Lercker, G. (2012). Oxidative Pattern from Fluorescent Light Exposition of Crystalline Cholesterol. Food Biophysics, 7(3), 209-219. doi:10.1007/s11483-012-9259-y

Medina-Meza, I. G., Rodriguez-Estrada, M. T., Lercker, G., Barnaba, C., & García, H. S. (2014). Kinetics of 25-hydroperoxycholesterol formation during photo-oxidation of crystalline cholesterol. Journal of the science of food and agriculture, 94(8), 1543-1551.

Meister, N., Aebischer, J., Vikas, M., Eyer, K., & De Pasquale, D. (2000). Spray-drying process. In: Google Patents.

Mellies, M., Glueck, C. J., Sweeney, C., Fallat, R. W., Tsang, R. C., & Ishikawa, T. T. (1976). Plasma and dietary phytosterols in children. Pediatrics, 57(1), 60-67.

Menéndez-Carreño, M., Varo, N., Mugueta, C., Restituto, P., Ansorena, D., & Astiasarán, I. (2011). Correlation between serum content of the main COPs (cholesterol oxidation products) from autoxidation and cardiovascular risk factors. Nutr Hosp, 26(1), 144-151.

Meynier, A., Andre, A., Lherminier, J., Grandgirard, A., & Demaison, L. (2005). Dietary oxysterols induce in vivo toxicity of coronary endothelial and smooth muscle cells. European journal of nutrition, 44(7), 393-405.

Micheletta, F., Natoli, S., Misuraca, M., Sbarigia, E., Diczfalusy, U., & Iuliano, L. (2004). Vitamin E supplementation in patients with carotid atherosclerosis: reversal of altered oxidative stress status in plasma but not in plaque. Arterioscler Thromb Vasc Biol, 24(1), 136-140. doi:10.1161/01.ATV.0000104028.07929.72

Min, J.-S., Lee, S.-O., Khan, M. I., Yim, D. G., Seol, K.-H., Lee, M., & Jo, C. (2015). Monitoring the formation of cholesterol oxidation products in model systems using response surface methodology. Lipids in health and disease, 14(1), 77.

Min, J. S., Khan, M. I., Lee, S. O., Yim, D. G., Seol, K. H., Lee, M., & Jo, C. (2016). Impact of Cooking, Storage, and Reheating Conditions on the Formation of Cholesterol Oxidation Products in Pork Loin. Korean J Food Sci Anim Resour, 36(1), 23-28. doi:10.5851/kosfa.2016.36.1.23

Miyoshi, N., Iuliano, L., Tomono, S., & Ohshima, H. (2014). Implications of cholesterol autoxidation products in the pathogenesis of inflammatory diseases. Biochemical and Biophysical Research Communications, 446(3), 702-708. doi:10.1016/j.bbrc.2013.12.107

Modzelewska-Kapituła, M., Pietrzak-Fiećko, R., Tkacz, K., Draszanowska, A., & Więk, A. (2019). Influence of sous vide and steam cooking on mineral contents, fatty acid composition and tenderness of semimembranosus muscle from Holstein-Friesian bulls. Meat Science, 157, 107877. doi:https://doi.org/10.1016/j.meatsci.2019.107877

Mohiuddin, A. K. (2019). Fast Food Addiction: A Major Public Health Concern.

Monteiro, C. A., Cannon, G., Lawrence, M., Costa Louzada, M. L., & Pereira Machado, P. (2019). Ultra-processed foods, diet quality, and health using the NOVA classification system. Rome

Monteiro, C. a. C. G. a. L. M. a. L. M. L. a. M. P. (2019). FAO. Ultra-processed foods, diet quality, and health using the NOVA classification system.

Moog, C., Deloulme, J. C., Baudier, J., Revel, M. O., Bischoff, P., Hietter, H., & Luu, B. (1991). Membrane-related oxysterol function: preliminary results on the modification of protein kinase C activity and substrate phosphorylation by 7ß,25-dihydroxycholesterol. Biochimie, 73(10), 1321-1326. doi:https://doi.org/10.1016/0300-9084(91)90096-J

Morais Júnior, W. G., Fernández-Lorente, G., Guisán, J. M., Ribeiro, E. J., De Resende, M. M., & Costa Pessela, B. (2017). Production of omega-3 polyunsaturated fatty acids through hydrolysis of fish oil by Candida rugosa lipase immobilized and stabilized on different supports. Biocatalysis and Biotransformation, 35(1), 63-73. doi:10.1080/10242422.2016.1268603

Moreau, R. A., Whitaker, B. D., & Hicks, K. B. (2002). Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses. Prog Lipid Res, 41(6), 457-500.

Mukhopadhyay, S., Fellows, K., Browne, R. W., Khare, P., Krishnan Radhakrishnan, S., Hagemeier, J., . . . Ramanathan, M. (2017). Interdependence of oxysterols with cholesterol profiles in multiple sclerosis. Mult Scler, 23(6), 792-801. doi:10.1177/1352458516666187

Murakami, H., Tamasawa, N., Matsui, J., Yamato, K., JingZhi, G., & Suda, T. (2001). Plasma levels of soluble vascular adhesion molecule-1 and cholesterol oxidation product in type 2 diabetic patients with nephropathy. J Atheroscler Thromb, 8(1), 21-24.

Neff, M. R., Bhavsar, S. P., Braekevelt, E., & Arts, M. T. (2014). Effects of different cooking methods on fatty acid profiles in four freshwater fishes from the Laurentian Great Lakes region. Food Chem, 164, 544-550. doi:10.1016/j.foodchem.2014.04.104

Nelson, E. R., Chang, C. Y., & McDonnell, D. P. (2014). Cholesterol and breast cancer pathophysiology. Trends Endocrinol Metab, 25(12), 649-655. doi:10.1016/j.tem.2014.10.001

Nestel, P., Cehun, M., Pomeroy, S., Abbey, M., & Weldon, G. (2001). Cholesterol-lowering effects of plant sterol esters and non-esterified stanols in margarine, butter and low-fat foods. Eur J Clin Nutr, 55, 1084–1090. doi:https://doi.org/10.1038/sj.ejcn.1601264

Nguyen, T. T. (1999). The Cholesterol-Lowering Action of Plant Stanol Esters. The Journal of Nutrition, 129(12), 2109-2112. doi:10.1093/jn/129.12.2109

Nielsen, J. H. (1995). Isolation and quantification of cholesterol oxides in dairy products by selected ion monitoring mass spectrometry. Journal of dairy research, 62(01).

Nielsen, J. H. (1996a). Cholesterol oxidation in butter and dairy spread during storage. Journal of dairy research, 63(01).

Nielsen, J. H. (1996b). Cholesterol oxidation in feta cheese produced from high-temperature bleached and from non-bleached butteroil from bovine milk. Journal of dairy research, 63(04).

NIH. (2017). High Blood Pressure. Retrieved from https://medlineplus.gov/highbloodpressure.html

NIH. (2021). PubChem. Retrieved from https://pubchem.ncbi.nlm.nih.gov/

Niot, I., Poirier, H., Tran, T. T. T., & Besnard, P. (2009). Intestinal absorption of long-chain fatty acids: Evidence and uncertainties. Progress in Lipid Research, 48(2), 101-115. doi:10.1016/j.plipres.2009.01.001

Normén, L., Ellegård, L., Brants, H., Dutta, P., & Andersson, H. (2007). A phytosterol database: Fatty foods consumed in Sweden and the Netherlands. Journal of Food Composition and Analysis, 20(3), 193-201. doi:https://doi.org/10.1016/j.jfca.2006.06.002

Nourooz-Zadeh, J. (1988). Cholesterol oxides in swedish foods and food ingredients: Butter and cheese. Journal of the American Oil Chemists' Society, 65(10), 1635-1641.

Novelli, E., Zanardi, E., Ghiretti, G. P., Campanini, G., Dazzi, G., Madarena, G., & Chizzolini, R. (1998). Lipid and cholesterol oxidation in frozen stored pork, salame Milano and mortadella. Meat Science, 48(1), 29 - 40. doi:https://doi.org/10.1016/S0309-1740(97)00072-7

O'Callaghan, Y., McCarthy, F. O., & O'Brien, N. M. (2014). Recent advances in Phytosterol Oxidation Products. Biochem Biophys Res Commun, 446(3), 786-791. doi:10.1016/j.bbrc.2014.01.148

Ogino, Y., Osada, K., Nakamura, S., Ohta, Y., Kanda, T., & Sugano, M. (2007). Absorption of dietary cholesterol oxidation products and their downstream metabolic effects are reduced by dietary apple polyphenols. Lipids, 42(2), 151.

Oje, J., Pazos, A. J., Martínez, D., Novoa, S., Sánchez, J. L., & Abad, M. (2004). Seasonal variation in weight and biochemical composition of the tissues of Ruditapes decussatus in relation to the gametogenic cycle. Aquaculture, 238(1), 451-468. doi:https://doi.org/10.1016/j.aquaculture.2004.05.022

Olkkonen, V. M., Béaslas, O., & Nissilä, E. (2012). Oxysterols and Their Cellular Effectors. Biomolecules, 2(1), 76.

Olkkonen, V. M., Gylling, H., & Ikonen, E. (2017). Plant sterols, cholesterol precursors and oxysterols: Minute concentrations—Major physiological effects. The Journal of steroid biochemistry and molecular biology, 169, 4-9.

Olson, C. L. (1976). On choosing a test statistic in multivariate analysis of variance. Psychological bulletin, 83(4), 579.

Osada, Kyoichi, Shinichi, H., Shingo, N., & Michihiro, S. (2000). Cholesterol Oxidation in Meat Products and Its Regulation by Supplementation of Sodium Nitrite and Apple Polyphenol before Processing. Journal of Agricultural and Food Chemistry, 48(9), 3823-3829. doi:10.1021/jf991187k

Osada, Kyoichi, Takehiro, K., Li, C., Koji, Y., & Michihiro, S. (1993). Levels and formation of oxidized cholesterol in processed marine foods. 41.

Osada, K., Sasaki, E., & Sugano, M. (1994). Inhibition of Cholesterol Absorption by Oxidized Cholesterol in Rats. Bioscience, Biotechnology, and Biochemistry, 58(4), 782-783. doi:10.1271/bbb.58.782

Ostlund, R., Racette, S., Okeke, A., & Stenson, W. (2002). Phytosterols that are naturally present in commercial corn oil significantly reduce cholesterol absorption in humans. The American journal of clinical nutrition, 75, 1000-1004. doi:10.1093/ajcn/75.6.1000

Ostlund, R., Racette, S., & Stenson, W. (2003). Inhibition of cholesterol absorption by phytosterol-replete wheat germ compared with phytosterol-depleted wheat germ1-3. The American journal of clinical nutrition, 77, 1385-1389. doi:10.1093/ajcn/77.6.1385

Ostlund, R. E. J. (2002). PHYTOSTEROLS IN HUMAN NUTRITION. Annual Review of Nutrition, 22(1), 533-549. doi:10.1146/annurev.nutr.22.020702.075220

Overholt, M. O., Mancini, S., Galloway, H. O., Preziuso, G., Dilger, A. C., & Boler, D. D. (2016). Effects of salt purity on lipid oxidation, sensory characteristics, and textural properties of fresh, ground pork patties. LWT - Food Science and Technology, 65, 890-896. doi:https://doi.org/10.1016/j.lwt.2015.08.067

Petrón, M. J. a. G.-R. J. A. a. M. L. a. M. E. a. A. T. (2003). Identification and Quantification of Cholesterol and Cholesterol Oxidation Products in Different Types of Iberian Hams. Journal of Agricultural and Food Chemistry, 51(19), 5786-5791. doi:10.1021/jf034100a

Pickett-Bernard, D. (2006). Book Review: Infant Formula: Evaluating the Safety of New Ingredients. Journal of Human Lactation, 22(2), 231-231. doi:10.1177/089033440602200221

Picouet, P. A., Fernandez, A., Serra, X., Sunol, J. J., & Arnau, J. (2007). Microwave heating of cooked pork patties as a function of fat content. Journal of Food Science, 72(2), E57-E63. doi:10.1111/j.1750-3841.2007.00269.x

Pie, J. E. a. S. K. a. S. C. (1991). Cholesterol oxidation in meat products during cooking and frozen storage. Journal of Agricultural and Food Chemistry, 39(2), 250-254. doi:10.1021/jf00002a005

Piironen, V., Toivo, J., & Lampi, A. M. (2000). Natural Sources of Dietary Plant Sterols. Journal of Food Composition and Analysis, 13(4), 619-624. doi:https://doi.org/10.1006/jfca.2000.0898

Pikul, J., Rudzińska, M., Teichert, J., Lasik, A., Danków, R., & Przybylski, R. (2013). Cholesterol oxidation during storage of UHT-treated bovine and caprine milk. International Dairy Journal, 30(1), 29-32. doi:10.1016/j.idairyj.2012.11.005

Pinedo, S., Vissers, M. N., Bergmann, K. v., Elharchaoui, K., Lütjohann, D., Luben, R., . . . Boekholdt, S. M. (2007). Plasma levels of plant sterols and the risk of coronary artery disease: the prospective EPIC-Norfolk Population Study. Journal of Lipid Research, 48(1), 139-144. doi:10.1194/jlr.M600371-JLR200

Prunet, C., Lemaire-Ewing, S., Ménétrier, F., Néel, D., & Lizard, G. (2005). Activation of caspase-3-dependent and-independent pathways during 7-ketocholesterol-and 7β-hydroxycholesterol-induced cell death: A morphological and biochemical study. Journal of biochemical and molecular toxicology, 19(5), 311-326.

Przygonski, K., Jelen, H., & Wasowicz, E. (2000). Determination of cholesterol oxidation products in milk powder and infant formulas by gas chromatography and mass spectrometry. Food / Nahrung, 44(2), 122-125. doi:10.1002/(SICI)1521-3803(20000301)44:2<122::AID-FOOD122>3.0.CO;2-R

Quality, A. f. H. R. a. (2020). 2019 NATIONAL HEALTHCARE

QUALITY AND DISPARITIES REPORT. Retrieved from https://www.ahrq.gov/sites/default/files/wysiwyg/research/findings/nhqrdr/2019qdr.pdf

Rajasekaran, A., & Kalaivani, M. (2013). Designer foods and their benefits: A review. J Food Sci Technol, 50(1), 1-16. doi:10.1007/s13197-012-0726-8

Ramírez, M., Amate, L., & Gil, A. (2001). Absorption and distribution of dietary fatty acids from different sources. Early Human Development, 65, S95-S101. doi:https://doi.org/10.1016/S0378-3782(01)00211-0

Rant, W., Radzik-Rant, A., Świątek, M., Niżnikowski, R., Szymańska, Ż., Bednarczyk, M., . . . Ślęzak, M. (2019). The effect of aging and muscle type on the quality characteristics and lipid oxidation of lamb meat. Arch Anim Breed, 62(2), 383-391. doi:10.5194/aab-62-383-2019

Raza, S., Meyer, M., Schommer, J., Hammer, K. D., Guo, B., & Ghribi, O. (2016). 27-Hydroxycholesterol stimulates cell proliferation and resistance to docetaxel-induced apoptosis in prostate epithelial cells. Med Oncol, 33(2), 12. doi:10.1007/s12032-015-0725-5

Rey, A., López-Bote, C., & Buckley, J. (2004). Effect of feed on cholesterol concentration and oxidation products development of longissimus dorsi muscle from Iberian pigs. Irish journal of agricultural and food research, 69-83.

Rodriguez-Estrada, M. T., Garcia-Llatas, G., & Lagarda, M. J. (2014). 7-Ketocholesterol as marker of cholesterol oxidation in model and food systems: when and how. Biochem Biophys Res Commun, 446(3), 792-797. doi:10.1016/j.bbrc.2014.02.098

Romeu-Nadal, M., Chavez-Servin, J. L., Castellote, A. I., Rivero, M., & Lopez-Sabater, M. C. (2007). Oxidation stability of the lipid fraction in milk powder formulas. Food Chemistry, 100(2), 756-763. doi:10.1016/j.foodchem.2005.10.037

Romina Pedreschi and Pablo Muñoz and Paula Robledo and Cecilia Becerra and Bruno, G. D. a. H. a. R. M. a. E. W. a. R. C. H. (2014). Metabolomics analysis of postharvest ripening heterogeneity of 'Hass' avocadoes. Postharvest Biology and Technology, 92, 172-179. doi:https://doi.org/10.1016/j.postharvbio.2014.01.024

Ros, E. (2000). Intestinal absorption of triglyceride and cholesterol. Dietary and pharmacological inhibition to reduce cardiovascular risk. Atherosclerosis, 151(2), 357-379.

Rose-Sallin, C. (1995). Quantification of Cholesterol Oxidation Products in Milk Powders Using [2H7]Cholesterol To Monitor Cholesterol Autoxidation Artifacts. Journal of agricultural and food chemistry, 43(4), 935-941.

Rossen, L. M., Simon, A. E., & Herrick, K. A. (2016). Types of Infant Formulas Consumed in the United States. Clin Pediatr (Phila), 55(3), 278-285. doi:10.1177/0009922815591881

Roussi, S., Winter, A., Gosse, F., Werner, D., Zhang, X., Marchioni, E., . . . Raul, F. (2005). Different apoptotic mechanisms are involved in the antiproliferative effects of 7beta-hydroxysitosterol and 7beta-hydroxycholesterol in human colon cancer cells. Cell Death Differ, 12(2), 128-135. doi:10.1038/sj.cdd.4401530

Rudzinska, M., Uchman, W., & Wasowicz, E. (2005). Plant

Sterols in Food Technology. Acta

Sci. Pol., Technol. Aliment, 4, 147–156.

Rutkowska, J., Adamska, A., Sinkiewicz, I., & Białek, M. (2012). Composition of fatty acids in selected sorts of biscuits, offered for children. Acta Alimentaria, 41(4), 433. doi:10.1556/aalim.41.2012.4.5

Ryan, E., Galvin, K., O'Connor, T. P., Maguire, A. R., & O'Brien, N. M. (2007). Phytosterol, squalene, tocopherol content and fatty acid profile of selected seeds, grains, and legumes. Plant Foods Hum Nutr, 62(3), 85-91. doi:10.1007/s11130-007-0046-8

Ryan, E., McCarthy, F. O., Maguire, A. R., & O'Brien, N. M. (2009). Phytosterol Oxidation Products: Their Formation, Occurrence, and Biological Effects. Food Reviews International, 25(2), 157-174. doi:10.1080/87559120802682797

Sander, B. D., Addis, P. B., Park, S. W., & Smith, D. E. (1989). Quantification of Cholesterol Oxidation-Products in a Variety of Foods. Journal of food protection, 52(2), 109-114.

Sarantinos, J. (1993). The identification and quantification of cholesterol oxides in foods. Deakin University,

Savage, G. P., Dutta, P. C., & Rodriguez-Estrada, M. T. (2002). Cholesterol oxides: their occurrence and methods to prevent their generation in foods. Asia Pacific Journal of Clinical Nutrition, 11(1), 72-78. doi:10.1046/j.1440-6047.2002.00270.x

Schulze, M. B., & Hu, F. B. (2002). Dietary patterns and risk of hypertension, type 2 diabetes mellitus, and coronary heart disease. Curr Atheroscler Rep, 4(6), 462-467. doi:10.1007/s11883-002-0051-1

Schött, H. F., Baumgartner, S., Husche, C., Luister, A., Friedrichs, S., Miller, C. M., . . . Lütjohann, D. (2017). Oxidation of sitosterol and transport of its 7-oxygenated products from different tissues in humans and ApoE knockout mice. J Steroid Biochem Mol Biol, 169, 145-151. doi:10.1016/j.jsbmb.2016.04.011

Schött, H. F., Husche, C., Friedrichs, S., Miller, C. M., McCarthy, F. O., Laufs, U., . . . Lütjohann, D. (2015). 7β-Hydroxysitosterol crosses the blood-brain barrier more favored than its substrate sitosterol in ApoE-/- mice. Steroids, 99(Pt B), 178-182. doi:10.1016/j.steroids.2015.03.006

Scopesi, F., Zunin, P., Mazzella, M., Testa, M., Boggia, R., Evangelisti, F., & Serra, G. (2002). 7-Ketocholesterol in human and adapted milk formulas. Clinical Nutrition, 21(5), 379 - 384. doi:https://doi.org/10.1054/clnu.2002.0556

Seet, R. C., Lee, C. Y., Lim, E. C., Quek, A. M., Yeo, L. L., Huang, S. H., & Halliwell, B. (2009). Oxidative damage in dengue fever. Free Radic Biol Med, 47(4), 375-380. doi:10.1016/j.freeradbiomed.2009.04.035

Sevanian, A., & Peterson, A. R. (1984). Cholesterol epoxide is a direct-acting mutagen. Proc Natl Acad Sci U S A, 81(13), 4198-4202.

Sevanian, A., & Peterson, A. R. (1986). The cytotoxic and mutagenic properties of cholesterol oxidation products. Food Chem Toxicol, 24(10-11), 1103-1110.

Shahzad, N., Khan, W., Shadab, M. D., Ali, A., Singh-Saluja, S., Sharma, S., . . . Al-Ghamdi, S. S. (2017). Phytosterols as a natural anticancer agent: Current status and future perspective. Biomedicine & Pharmacotherapy, 88, 786-794. doi:https://doi.org/10.1016/j.biopha.2017.01.068

Shan, Z., Rehm, C. D., Rogers, G., Ruan, M., Wang, D. D., Hu, F. B., . . . Bhupathiraju, S. N. (2019). Trends in Dietary Carbohydrate, Protein, and Fat Intake and Diet Quality Among US Adults, 1999-2016. JAMA, 322(12), 1178-1187. doi:10.1001/jama.2019.13771

Shim, C. Y., Park, S., Choi, E. Y., Kang, S. M., Cha, B. S., Ha, J. W., . . . Chung, N. (2008). Is albuminuria an indicator of myocardial dysfunction in diabetic patients without overt heart disease? A study with Doppler strain and strain rate imaging. Metabolism, 57(4), 448-452. doi:10.1016/j.metabol.2007.11.003

Shori, A. B., Albaik, M., & Bokhari, F. M. (2017). Fast food consumption and increased body mass index as risk factors for weight gain and obesity in Saudi Arabia. Obesity Medicine, 8, 1-5. doi:https://doi.org/10.1016/j.obmed.2017.09.002

Shoshana Rozner and Nissim, G. (2006). The activity and absorption relationship of cholesterol and phytosterols. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 282-283, 435-456. doi:https://doi.org/10.1016/j.colsurfa.2005.12.032

Shozen, K.-i. (1995). Formation of Cholesterol Oxides in Marine Fish Products Induced by Grilling. Fisheries science, 61(5), 817-821.

Sieber, R. (2005). Oxidised cholesterol in milk and dairy products. International Dairy Journal, 15(3), 191-206. doi:10.1016/j.idairyj.2004.07.013

Sinéad Lordan and John, J. M. a. N. M. O. B. (2009). Oxysterols and mechanisms of apoptotic signaling: implications in the pathology of degenerative diseases. The Journal of Nutritional Biochemistry, 20(5), 321-336. doi:https://doi.org/10.1016/j.jnutbio.2009.01.001

Sirot, V. a. O. M. a. B. N. a. V. J.-L. a. L. J.-C. (2008). Lipid and fatty acid composition of fish and seafood consumed in France: CALIPSO study. Journal of Food Composition and Analysis, 21, 8-16. doi:10.1016/j.jfca.2007.05.006

Smith, B. (1985). The role of Chenopodium as a domesticate in pre-maize garden systems of the

eastern United State. Southeastern Archaeology, 4, 51-72.

Smith, L. L. (1996). Review of progress in sterol oxidations: 1987-1995. Lipids, 31(5), 453-487. doi:10.1007/bf02522641

Snelson, M., Tan, S. M., Clarke, R. E., de Pasquale, C., Thallas-Bonke, V., Nguyen, T. V., . . . Coughlan, M. T. (2021). Processed foods drive intestinal barrier permeability and microvascular diseases. Sci Adv, 7(14). doi:10.1126/sciadv.abe4841

Song, J., Park, J., Jung, J., Lee, C., Gim, S. Y., Ka, H., . . . Lee, J. (2015). Analysis of Trans Fat in Edible Oils with Cooking Process. Toxicol Res, 31(3), 307-312. doi:10.5487/TR.2015.31.3.307

Sooah Kim and Jungyeon Kim and Eun Ju Yun and Kyoung Heon, K. (2016). Food metabolomics: from farm to human. Current Opinion in Biotechnology, 37, 16-23. doi:https://doi.org/10.1016/j.copbio.2015.09.004

Su, M. Y., Broadhurst, M., Liu, C. P., Gathercole, J., Cheng, W. L., Qi, X. Y., . . . Haigh, B. (2017). Comparative analysis of human milk and infant formula derived peptides following in vitro digestion. Food Chem, 221, 1895-1903. doi:10.1016/j.foodchem.2016.10.041

Sun, C., Wei, W., Su, H., Zou, X., & Wang, X. (2018). Evaluation of sn-2 fatty acid composition in commercial infant formulas on the Chinese market: A comparative study based on fat source and stage. Food Chemistry, 242, 29-36. doi:https://doi.org/10.1016/j.foodchem.2017.09.005

Sun, X. D., & Holley, R. A. (2010). High Hydrostuatic Pressure Effects on the Texture of Meat and Meat Products. Journal of Food Science, 75(1), R17-R23. doi:10.1111/j.1750-3841.2009.01449.x

Tai, C. Y., Chen, Y. C., & Chen, B. H. (1999). Analysis, formation and inhibition of cholesterol oxidation products in foods: An overview (Part I). Journal of Food and Drug Analysis, 7(4), 243-257.

Tamanna, N., & Mahmood, N. (2015). Food Processing and Maillard Reaction Products: Effect on Human Health and Nutrition. Int J Food Sci, 2015, 526762. doi:10.1155/2015/526762

Tara Grauwet and Liesbeth Vervoort and Ines Colle and Ann and Marc, H. (2014). From fingerprinting to kinetics in evaluating food quality changes. Trends in Biotechnology, 32(3), 125-131. doi:https://doi.org/10.1016/j.tibtech.2014.01.002

Tarvainen, M., Nuora, A., Quirin, K., Kallio, H., & Yang, B. (2015). Effects of CO2 plant extracts on triacylglycerol oxidation in Atlantic salmon during cooking and storage. Food Chemistry, 173, 1011-1021. doi:https://doi.org/10.1016/j.foodchem.2014.10.125

Tavella, M., Peterson, G., Espeche, M., Cavallero, E., Cipolla, L., Perego, L., & Caballero, B. n. (2000). Trans fatty acid content of a selection of foods in Argentina. Food Chemistry, 69(2), 209-213. doi:https://doi.org/10.1016/S0308-8146(99)00257-5

TMIC. (2021). Human Metabolome Database. Retrieved from https://hmdb.ca/

Tomoyori, H., Kawata, Y., Higuchi, T., Ichi, I., Sato, H., Sato, M., . . . Imaizumi, K. (2004). Phytosterol oxidation products are absorbed in the intestinal lymphatics in rats but do not accelerate atherosclerosis in apolipoprotein E-deficient mice. J Nutr, 134(7), 1690-1696. doi:10.1093/jn/134.7.1690

Tracie Seimon and Ira, T. (2009). Mechanisms and consequences of macrophage apoptosis in atherosclerosis. Journal of Lipid Research, 50, S382-S387. doi:https://doi.org/10.1194/jlr.R800032-JLR200

Traves, D. (2019). Understanding infant formula. Paediatrics and Child Health, 29(9), 384-388. doi:https://doi.org/10.1016/j.paed.2019.06.003

Trevisi, L., Bertoldo, A., Agnoletto, L., Poggiani, C., Cusinato, F., & Luciani, S. (2010). Antiapoptotic and Proliferative Effects of Low Concentrations of 7β-Hydroxycholesterol in Human Endothelial Cells via ERK Activation.

Tsai, L. S., & Hudson, C. A. (1984). Cholesterol Oxides in Commercial Dry Egg Products: Quantitation. Journal of Food Science, 50(1), 229-231. doi:10.1111/j.1365-2621.1985.tb13316.x

Tulve, N. S., Egeghy, P. P., Fortmann, R. C., Xue, J. P., Evans, J., Whitaker, D. A., & Croghan, C. W. (2011). Methodologies for estimating cumulative human exposures to current-use pyrethroid pesticides. Journal of Exposure Science and Environmental Epidemiology, 21(3), 317-327. doi:10.1038/jes.2010.25

Uauy, R., & Dangour, A. D. (2009). Fat and fatty acid requirements and recommendations for infants of 0-2 years and children of 2-18 years. Ann Nutr Metab, 55(1-3), 76-96. doi:10.1159/000228997

Ubhayasekera, S., Kochhar, S. P., & Dutta, P. C. (2006). Lipids and lipid oxidation with emphasis on cholesterol oxides in some Indian sweets available in London. International Journal of Food Sciences and Nutrition, 57(7-8), 451-458. doi:10.1080/09637480600888834

Upston, J. M., Niu, X., Brown, A. J., Mashima, R., Wang, H., Senthilmohan, R., . . . Stocker, R. (2002). Disease stage-dependent accumulation of lipid and protein oxidation products in human atherosclerosis. Am J Pathol, 160(2), 701-710. doi:10.1016/S0002-9440(10)64890-0

USDA. (2016). Ground Beef and Food Safety. Retrieved from https://www.fsis.usda.gov/food-safety/safe-food-handling-and-preparation/meat/ground-beef-and-food-safety#:~:text=Beef%20fat%20may%20be%20added,%2C%20extenders%2C%20or%20binders%20added.

USDA. (2018). Butter. Retrieved from https://fdc.nal.usda.gov/fdc-app.html#/food-details/1605291/nutrients

USDA. (2019a). Beef, rib, large end (ribs 6-9), separable lean and fat, trimmed to 0" fat, choice, cooked, roasted. Retrieved from https://fdc.nal.usda.gov/fdc-app.html#/food-details/170619/nutrients

USDA. (2019b). French fries - McDOnald's.

USDA. (2019c). Hotcakes - McDonald's.

USDA. (2019d). KFC, biscuit. Retrieved from https://fdc.nal.usda.gov/fdc-app.html#/food-details/170339/nutrients

USDA. (2020). Fish sandwich, fried, from fast food, with cheese. Retrieved from https://fdc.nal.usda.gov/fdc-app.html#/food-details/1099897/nutrients

Valérie Riendeau and Christophe, G. Effect of 27-hydroxycholesterol on survival and death of human macrophages and vascular smooth muscle cells. Free Radical Research, 43(10), 1019-1028. doi:10.1080/10715760903040610

Van Den Brenk, J., Van Dijke, K. C., Van Der Steen, A. M. L., Moonen, R. C. J., & Van Baalen, A. (2015). Google Patents.

Van Dijke, K. C., SCHRÖDER, J., USTUNEL, M. A., Reinhold HALSEMA, D. G., & Moonen, R. C. J. (2018).

Vandenplas, Y., Zakharova, I., & Dmitrieva, Y. (2015). Oligosaccharides in infant formula: more evidence to validate the role of prebiotics. Br J Nutr, 113(9), 1339-1344. doi:10.1017/s0007114515000823

Vaughn, D. W., Green, S., Kalayanarooj, S., Innis, B. L., Nimmannitya, S., Suntayakorn, S., . . . Nisalak, A. (1997). Dengue in the early febrile phase: viremia and antibody responses. J Infect Dis, 176(2), 322-330.

Vaya, J., Aviram, M., Mahmood, S., Hayek, T., Grenadir, E., Hoffman, A., & Milo, S. (2001). Selective distribution of oxysterols in atherosclerotic lesions and human plasma lipoproteins. Free Radic Res, 34(5), 485-497.

Vejux, A., & Lizard, G. (2009). Cytotoxic effects of oxysterols associated with human diseases: Induction of cell death (apoptosis and/or oncosis), oxidative and inflammatory activities, and phospholipidosis. Molecular aspects of medicine, 30(3), 153-170.

Vejux, A., & Lizard, G. (2009). Cytotoxic effects of oxysterols associated with human diseases: Induction of cell death (apoptosis and/or oncosis), oxidative and inflammatory activities, and phospholipidosis. Mol Aspects Med, 30(3), 153-170. doi:10.1016/j.mam.2009.02.006

Verardo, V., Riciputi, Y., Messia, M. C., Marconi, E., & Caboni, M. F. (2016). Influence of drying temperatures on the quality of pasta formulated with different egg products. European Food Research and Technology, 243(5), 817-825. doi:10.1007/s00217-016-2795-9

Vergères, G. (2013). Nutrigenomics – Linking food to human metabolism. Trends in Food Science & Technology, 31(1), 6-12. doi:https://doi.org/10.1016/j.tifs.2013.02.002

Verhé, R., Verleyen, T., Hoed, V., & Greyt, W. (2005). Influence of refining of vegetable oils on minor components.

Virginio, V. W., Nunes, V. S., Moura, F. A., Menezes, F. H., Andreollo, N. A., Rogerio, F., . . . Sposito, A. C. (2015). Arterial tissue and plasma concentration of enzymatic-driven oxysterols are associated with severe peripheral atherosclerotic disease and systemic inflammatory activity. Free Radic Res, 49(2), 199-203. doi:10.3109/10715762.2014.992894

Vore, V. R. (1988). TBA Values and 7-Ketocholesterol in Refrigerated Raw and Cooked Ground Beef. Journal of Food Science, 53(4), 1058-1061. doi:10.1111/j.1365-2621.1988.tb13529.x

Wang, T. Y., Liu, M., Portincasa, P., & Wang, D. Q. H. (2013). New insights into the molecular mechanism of intestinal fatty acid absorption. European Journal of Clinical Investigation, 43(11), 1203-1223. doi:10.1111/eci.12161

Wolf, B., & Green, D. (1999). Suicidal Tendencies: Apoptotic Cell Death by Caspase Family Proteinases*. Journal of Biological Chemistry, 274(29), 20049-20052. doi:https://doi.org/10.1074/jbc.274.29.20049

Wood, M. E. a. K. H. a. B. H. a. G. A. J. F. a. J. D. (1996). Fatty acid content and composition of english beef, lamb and pork at retail. Meat Science, 42(4), 443-456. doi:https://doi.org/10.1016/0309-1740(95)00037-2

Wu, Q., Ishikawa, T., Sirianni, R., Tang, H., McDonald, J. G., Yuhanna, I. S., . . . Shaul, P. W. (2013). 27-Hydroxycholesterol promotes cell-autonomous, ER-positive breast cancer growth. Cell Rep, 5(3), 637-645. doi:10.1016/j.celrep.2013.10.006

Xu, L., Korade, Z., Rosado, D. A., Liu, W., Lamberson, C. R., & Porter, N. A. (2011). An oxysterol biomarker for 7-dehydrocholesterol oxidation in cell/mouse models for Smith-Lemli-Opitz syndrome. J Lipid Res, 52(6), 1222-1233. doi:10.1194/jlr.M014498

Xu, L., Mirnics, K., Bowman, A. B., Liu, W., Da, J., Porter, N. A., & Korade, Z. (2012). DHCEO accumulation is a critical mediator of pathophysiology in a Smith–Lemli–Opitz syndrome model. Neurobiology of Disease, 45(3), 923-929. doi:https://doi.org/10.1016/j.nbd.2011.12.011

Xue, J., Zartarian, V., Dang, W., Graham, G., Smith, L., & Stallings, C. (2006). A Probabilistic Arsenic Exposure Assessment for Children Who Contact Chromated Copper Arsenate (CCA)-Treated Playsets and Decks, Part 2: Sensitivity and Uncertainty Analyses. Risk analysis : an official publication of the Society for Risk Analysis, 26, 533-541. doi:10.1111/j.1539-6924.2006.00748.x

Xue, J. P., Zartarian, V., Wang, S. W., Liu, S. V., & Georgopoulos, P. (2010). Probabilistic Modeling of Dietary Arsenic Exposure and Dose and Evaluation with 2003-2004 NHANES Data. Environmental Health Perspectives, 118(3), 345-350. doi:10.1289/ehp.0901205

Xue, J. P., Zartarian, V. G., Liu, S. V., & Geller, A. M. (2012). Methyl mercury exposure from fish consumption in vulnerable racial/ethnic populations: Probabilistic SHEDS-Dietary model analyses using 1999-2006 NHANES and 1990-2002 TDS data. Science of the Total Environment, 414, 373-379. doi:10.1016/j.scitotenv.2011.10.010

Yarmand, M. S., & Homayouni, A. (2009). Effect of microwave cooking on the microstructure and quality of meat in goat and lamb. Food Chemistry, 112(4), 782-785. doi:10.1016/j.foodchem.2008.06.033

Yoshida, T., Matsuzaki, Y., Haigh, W. G., Fukushima, S., Ikezawa, K., Tanaka, N., & Lee, S. P. (2003). Origin of oxysterols in hepatic bile of patients with biliary infection. Am J Gastroenterol, 98(10), 2275-2280. doi:10.1111/j.1572-0241.2003.07703.x

Zardetto, S., Barbanti, D., & Rosa, M. D. (2014). Formation of cholesterol oxidation products (COPs) and loss of cholesterol in fresh egg pasta as a function of thermal treatment processing. Food Research International, 62, 177-182. doi:https://doi.org/10.1016/j.foodres.2014.02.028

Zarrouk, A., Vejux, A., Mackrill, J., O'Callaghan, Y., Hammami, M., O'Brien, N., & Lizard, G. (2014). Involvement of oxysterols in age-related diseases and ageing processes. Ageing Res Rev, 18, 148-162. doi:10.1016/j.arr.2014.09.006

Zartarian, V., Xue, J., Özkaynak, H., Dang, W., Glen, G., Smith, L., & Stallings, C. (2006). A Probabilistic Arsenic Exposure Assessment for Children who Contact CCA-Treated Playsets and Decks, Part 1: Model Methodology, Variability Results, and Model Evaluation. Wiley-Blackwell: Risk Analysis (Archive).

Zartarian, V., Xue, J. P., Glen, G., Smith, L., Tulve, N., & Tornero-Velez, R. (2012). Quantifying children's aggregate (dietary and residential) exposure and dose to permethrin: application and evaluation of EPA's probabilistic SHEDS-Multimedia model. Journal of Exposure Science and Environmental Epidemiology, 22(3), 267-273. doi:10.1038/jes.2012.12

Zartarian, V. G., Ozkaynak, H., Burke, J. M., Zufall, M. J., Rigas, M. L., & Furtaw, E. J. (2000). A modeling framework for estimating children's residential exposure and dose to chlorpyrifos via dermal

residue contact and nondietary ingestion. Environmental Health Perspectives, 108(6), 505-514. doi:10.2307/3454611

Zinocker, M. K., & Lindseth, I. A. (2018). The Western Diet-Microbiome-Host Interaction and Its Role in Metabolic Disease. Nutrients, 10(3). doi:10.3390/nu10030365

Zubillaga M. P. and Maerker, G. (1991). Quantification of Three Cholesterol Oxidation Products in Raw Meat and Chicken. Journal of Food Science, 56(5), 1194-1196. doi:10.1111/j.1365-2621.1991.tb04732.x

Zunin, P., Boggia, R., & Evangelisti, F. (2001). Identification and quantification of cholesterol oxidation products in canned tuna. Journal of the American Oil Chemists' Society, 78(10), 1037-1040. doi:10.1007/s11746-001-0384-y

Zunin, P., Calcagno, C., & Evangelisti, F. (1998). Sterol oxidation in infant milk formulas and milk cereals. J Dairy Res, 65(4), 591-598.