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CHOLESTEROL OXIDATION IN WHOLE MILK POWDER AS
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CHOLESTEROL OXIDATION IN WHOLE MILK POWDER AS INFLUENCED BY PROCESSING AND PACKAGING

BY

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ABSTRACT

CHOLESTEROL OXIDATION IN WHOLE MILK POWDER AS INFLUENCED BY PROCESSING AND PACKAGING

BY

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This study was designed to examine the effects of various spray-drying processes (direct firing, high levels of oxides of nitrogen (NO_x); direct firing, low NO_x; indirect electric heating) and packaging systems (polyethylene pouches and crimp-sealed glass vials, with and without oxygen absorbers) on the oxidative stability of lipids, including cholesterol, during the storage of whole milk powders. Lipid oxidation, including the generation of cholesterol oxidation products, was greatest in samples processed by high NO_x direct-fired dryers. Oxygen absorbers effectively controlled cholesterol oxidation during the entire storage period, even in those samples from the high NO_x drying system. There was a positive correlation (r= +0.89) between the extent of lipid oxidation and cholesterol oxidation in all the samples. It was concluded that the stability of whole milk powder during storage can be increased by using low NO_x drying processes and by packaging in oxygen-impermeable packages containing oxygen absorbers.

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TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

INTRODUCTION

Cholesterol is susceptible to oxidation in the presence of oxygen, heat, light and radiation. Some of the cholesterol oxidation products (COPs) have been implicated in adverse biological effects, e.g., atherogenesis, cytotoxicity, carcinogenesis and inhibition of cholesterol synthesis (Kumar and Singhal, 1991).

Foods of animal origin may contain varying concentrations of COPs, depending upon the severity of processing or storage conditions. Whole milk powders contain 0.2 to 0.4 % cholesterol and when subjected to various thermal treatments and storage at ambient temperature for prolonged periods, may undergo oxidation. Until recently, however, cholesterol oxidation in whole milk powders has not received much attention in comparison to comparable studies with egg products.

The author hypothesizes that the formation of COPs in whole milk powder may be eliminated or reduced by developing better methods of processing, packaging, and storage of the products. Recently, the influence of spray drying method on the formation of cholesterol oxides in egg powder has been examined (Tsai and Hudson, 1985; Missler et al., 1985). It was demonstrated that the increase in the levels of nitrogen oxides (NO_x) generated during the spray drying process, enhances the oxidation of cholesterol in egg powder.

The major objective of this study was to examine the influence of three methods of spray drying (indirect, electric; direct, low NO_x; direct, high NO_x) on the formation of cholesterol oxides in whole milk powder. In addition, the oxidative changes of milkfat and cholesterol in whole milk powders during storage under different conditions were also evaluated.

Another objective of the study was to evaluate various packaging systems in suppressing lipid oxidation in whole milk powders during storage. The effect of three different packaging systems (polyethylene pouches, crimp-on glass vials with or without oxygen absorbers) on the storage stability of whole milk powders was determined.

REVIEW OF LITERATURE

Methods of Drying

Powdered milk production is an important segment of the dairy industry. Milk powders provide not only a means of handling excess milk produced for other dairy products, especially market milk, but also provide better keeping quality, less storage space, and lower shipping costs. As a result of dry milk production, it is possible to make milk available in regions that are not suitable for dairying, or where the milk production is insufficient.

Milk or milk products can be dried in several ways: 1) roller drying, 2) spray drying, 3) freeze drying, 4) vacuum drying, 5) foam mat drying, and 6) by a vortex method (Hall and Hedrick, 1966). Roller drying and spray drying are the major methods of producing milk solids for milk and milk products. However, roller drying does not meet today's requirements with regard to powder quality, high capacity, high preconcentration and low operating and maintenance costs. Therefore, spray drying has become the most important method in dry milk production (Knipschildt, 1986).

The spray dryer system is somewhat complicated. According to the method of furnishing heat and the method of heating the air, spray driers can be classified as: 1) direct-fired spray driers -- burning gas or fuel oil and the products of combustion heating the air directly; 2) indirect-fired spray driers -- using steam or electricity as heat sources and the heat being transferred across heat exchanger plates or coils to the air (Hall and Hedrick, 1966). Most spray driers tend to be the indirect type as a result of the better quality of the products produced, and the relatively nonhazardous nature of the heat source. However, the direct-fired units are more efficient due to less heat loss during heat transfer. Further, the capital investment and maintenance costs in a direct system are substantially less than for an indirect system. For these reasons, there is a growing interest in the use of direct-fired heating systems to produce dried milk products (Kelly and Slattery, 1985; Jansen and Elgersma, 1985; Kelly et al., 1989), although they are not yet widely used in the food industry.

With a direct-fired unit, the selection of fuel is based primarily on the cost and the effect of fuel combustion products on the drying product. In recent years, the availability of natural gas and high fuel prices have created considerable interest in the direct gas-fired burner. However, the danger of contaminating milk powder by products arising from direct gas firing has also been recognized (Knipschildt, 1986). The contamination is produced by oxides of nitrogen, e.g., nitrogen monoxide (NO) and nitrogen dioxide (NO_2) (commonly known as NO_x gases), in the combustion gases. During dehydration, these gases dissolve in the moist atmosphere of the drier to form nitric and nitrous acids, and contribute to the nitrate and nitrite contents in milk powders. Research has shown that the nitrite and nitrate contents in milk powders prepared in a direct gas-fired dryer, can be as much as double those present in powders produced by indirect heating (Harding and Gregson, 1978). N-Nitrosamines may also be present in the products and are formed from the reaction of nitrite with amines associated with the proteins of the products (Libbey et al., 1980; Challis et al., 1982; Havery et al., 1982).

It has been shown that when direct gas-fired spray dryers are used, nitrite, nitrate and N-nitrosamines are formed in the powder at a rate directly proportional to the amount of nitrogen oxides in the drying air (Kinpschildt, 1986). In order to prevent the formation of these undesirable compounds in food products, many researches have attempted to minimize contamination of the drying air by the combustion products in the flue gases from the burner. Rothery (1968) found that the nitrite contents of skim milk powder and sodium caseinate could be significantly reduced by injection of steam into the flame when direct gas-firing is used. However, a burner using steam injection to obtain lower NO_x levels could raise the moisture content in the dried products, and may offset some of the energy savings associated with direct firing. Therefore, special burners which produce minimum amounts of combustion-generated NO_x are required.

Wheeler (1980) summarized the mechanism of NO formation from air as a result of combustion. According to his theory, the rate of thermal formation of NO from air is strongly temperature-dependent and does not take place by a simple combination of nitrogen and oxygen, but through a set of inter-related reactions in which the predominantly active reactants are atomic oxygen, atomic nitrogen and hydroxyl radicals.

$N_2 + O^{\cdot} \text{ ----> } NO + N^{\cdot}$

$O_2 + N^{\cdot} \text{ ----> } NO + O^{\cdot}$

$OH + N^{\cdot} \text{ ----> } NO + H^{\cdot}$

At flame temperatures in the range 1500 to 1600°C, the formation of NO starts to be significant. The transformation of nitrogen monoxide to nitrogen dioxide is a low temperature reaction which takes place at approximately 600°C. Therefore, it was suggested that the combustion requirements for minimizing the formation of NO_x are low burning temperatures, but high enough to ensure complete combustion, and a short duration of the top temperature (Knipschildt, 1986) .

Conventional gas burners will produce an NO_x level of between 50 parts per million (ppm) and several hundred ppm (typically about 150 ppm) (Knipschildt, 1986). In 1982, Altermark and Hess developed a low-NO_x burner which could reduce the NO_x level to 2-6 ppm. The low-NO_x burner incorporated several important features: (1) flame cooling by means of inert or recycled flue gases; (2) homogeneous premixing of fuel gas, combustion air and cooling gas to prevent temperature peaks during combustion; and (3) an almost adiabatic environment of the flame to prevent temperature gradients and to ensure complete combustion at low temperature. The studies of Altermark and Hess (1982) clearly demonstrated the benefits resulting from reduced levels of combustion-generated nitrogen oxides, and this led to the development of the CXA low-NO_x gas burner (Urquhart Engineering Co. Ltd, England). This burner uses high excess air levels to burn the gas at the lower limit of its inflammability. Thus, the combustion temperature is lower and the duration of the top temperature in the flame is minimized. To ensure the absence of pockets of gas mixture, the combustion of which would result in localized high temperatures and high NO_x levels, gas and air are premixed using a multi-venturi gas/air mixer. Because of the slower flame speeds associated with combustion at high excess air levels, specially designed flame stabilizers are used to enhance flame stability. The combustion chamber is refractory-lined in order to increase flame stability by reradiation and also to ensure combustion completeness before the quenching of the combustion products by the air to be heated. A diagram of the CXA low-NO_x gas burner is shown in Appendix 1.

A previous study (Kelly et al., 1989) on the use of the low-NO_x CXA burner for milk drying has shown that the NO_x

levels at the burner throat were in the range of 1.0 to 1.65 μl/l which are superior to those reported by Jansen and Elgersma (1985). Moreover, there was no significant difference between the nitrate contents of skim milk powders produced by direct firing, electrical heating, and commercial producers (indirect heating), and the levels of N-nitrosodimethylamine (NDMA) were unaffected. Only small increases (0.2-1.7 ppm) in the concentrations of nitrite were detected.

Mechanism of Lipid Oxidation

Lipid oxidation is a major deteriorative reaction that can occur in foods during storage. It is responsible for a wide variety of undesirable reactions such as flavor and color changes, loss of nutritive value as a result of the reaction of oxidation products with proteins, and possible adverse biological effects (Addis, 1986). To prevent the onset of oxidation, an understanding of the initiation of oxidative changes in foods is necessary.

As a result of the many investigations over the past fifty years, the fundamental mechanisms of lipid oxidation are well established (Korycka-Dahl and Richardson, 1980; Nawar, 1985; Chan, 1987). However, many details of the reaction and its consequences remain unknown.

Lipid oxidation proceeds via a free-radical chain reaction which can be divided into three separate steps as indicated below:

Initiation -- formation of free radicals

$$RH \text{ -------------> } R^{\cdot} + H^{\cdot}$$

$$RH + O_2 \text{ ---------> } ROO^{\cdot} + H^{\cdot}$$

Propagation -- the free radical chain reaction

$$R^{\cdot} + O_2 \text{ ---------> } ROO^{\cdot}$$

$$ROO^{\cdot} + RH \text{ -------> } ROOH + R^{\cdot}$$

Termination -- formation of non-reactive products

$$R^{\cdot} + R^{\cdot} \text{ ----------> } RR$$

$$R^{\cdot} + ROO^{\cdot} \text{ --------> } ROOR$$

$$ROO^{\cdot} + ROO^{\cdot} \text{ ------> } ROOR + O_2$$

where RH refers to any unsaturated fatty acid in which the H is labile due to the activating influence of the adjacent double bond. $R^{\cdot}$ and $ROO^{\cdot}$ refer to the lipid alkyl and peroxy radicals, respectively.

The rate of oxidation of fatty acids is dependent on the degree of unsaturation. The reaction can be accelerated by pro-oxidant factors such as metals, free radicals, light, elevated temperature and moisture. Activated oxygen species, including singlet oxygen, hydroxyl radical, ozone, superoxide anion and hydrogen peroxide, may be important in initiating oxidative changes in foods. Rawls and Van Santen (1970) indicated that unless a catalyst is involved, singlet oxygen ($^1O_2$) is believed to be responsible for initiation. Once the reaction has been initiated, hydroperoxides (ROOH) are formed and subsequently undergo homolytic cleavage, forming two radicals which induce the autocatalytic propagation of the oxidative mechanism and yield a wide variety of secondary products. Termination reactions occur when the concentration of radicals are sufficiently great to permit kinetically preferential radical-radical interactions. The final products of oxidation, although dependent on the type of oxidized fat or oil, generally include short chain aldehydes,ketones, acids, alcohols and other carbonyl compounds (Nawar, 1985).

Stability of Whole Milk Powder during Storage

Composition of milkfat

Milkfat consists of approximately 95-96% triacylglycerols, 1.3-1.6% diacylglycerols, and 0.8-1.0% phospholipids. It also contains varying quantities of other compounds such as monoacylglycerols, carotenoids, free fatty acids, sterols and vitamins (Kurtz, 1974). The major fractions of bovine milk lipids are listed in Table 1.

Fatty acids, the major components of triacylglycerols in milkfat, account for over 85% of the total weight. The relative proportions of the various fatty acids in milkfat are affected by many factors such as feeding conditions and seasonal variations. Milkfat with a linoleic acid content as high as 30 % has been reported by Hill et al. (1977). Approximately 500 fatty acids have been detected in milkfats

(Sonntag, 1979). It is probable that additional fatty acids remain to be identified. The major fatty acids in bovine milkfat are listed in Table 2.

Milkfat is distinguished from other fats by the low average molecular weight of its fatty acids and by its high content of steam volatile acids, including butyric acid, which is unique to milkfat from ruminants. Milkfat contains predominantly saturated fatty acids ranging from C_4 to C_{18}. The unsaturated fatty acids of milkfat are mainly monounsaturated. Oleic acid ($C_{18:1}$) is the principal unsaturated fatty acid, with small amounts of di-unsaturated fatty acids, principally $C_{18:2}$ (linoleic acid) and trace amounts of other polyunsaturated fatty acids. For lipid oxidation, the important lipids in foods are the unsaturated fatty acids, particularly oleate, linoleate, and linolenate (Labuza, 1971). The susceptibility and rate of oxidation of these fatty acids increase with their degree of unsaturation. Therefore, measuring the extent of oxidation of these fatty acids can help determine the point at which rancidity occurs.

Most fatty acids of milkfat are present in triacylglycerols, but small proportions of free fatty acids are always present in fresh milkfat. Larger percentages of free fatty acids in milk are found in samples stored over time. Ritchie (1967) found little or no qualitative difference between the fatty acid profiles of free and total fat in whole milk powder.

Table 1. The major fractions of bovine milk lipids.

Component	% Total milk lipids
Triacylglycerols	95-96
Diacylglyerols	1.26-1.59
Monoacylglycerols	0.016-0.038
Phospholipids (total)	0.8-1.0
Sterols	0.22-0.41
Free fatty acids	0.10-0.44

Adapted from Kurtz (1974).

Table 2. The major fatty acids in bovine milkfat[a]

Fatty acid	Wt %	Fatty acid	Wt %
$C_{4:0}$	2.79	$C_{15:0}$	0.79
$C_{6:0}$	2.34	$C_{16:0}$	23.8
$C_{8:0}$	1.06	$C_{16:1}$	1.78
$C_{10:0}$	3.04	$C_{17:0}$	0.7
$C_{10:1}$	0.27	$C_{18:0}$	13.2
$C_{12:0}$	2.87	$C_{18:1}$	29.63
$C_{14:0}$	8.94	$C_{18:2}$	2.85
$C_{14:1}$	0.75	$C_{18:3}$	0.38

[a]Adapted from Kurtz (1974)

Oxidation of milkfat

Dry whole milk has a shelf life of less than 6 months, mainly as a result of its susceptibility to oxidation (Boon et al., 1976). The oxidation of fat in whole milk powder has recently been reviewed by Tuohy (1987). Oxidation of milkfat occurs according to the classical mechanism. However, the complex composition of dairy products as well as processing , manufacturing, and storage conditions, tend to influence both the rate of oxidation and the composition and percentage of oxidation products formed. In whole milk powder, the triacylglycerols are relatively susceptible to oxidation, whereas the phospholipids are more stable (Patton, 1962). Conversely, in fluid milk, phospholipids will undergo oxidation more readily than the triacylglycerols (Lea, 1953).

The fatty acids in milkfat are mainly saturated. From a practical viewpoint, the oxidation of saturated fatty acids at ambient temperatures can be ignored, as they remain unchanged relative to the unsaturated fatty acids (Nawar, 1985). Milkfat contains unsaturated fatty acids, e.g., oleic acid, linoleic acid and linolenic acid, and these are susceptible to oxidation. Pathways for the formation of hydroperoxides from these acids have been outlined by Frankel (1962).

Oleic acid, a major unsaturated fatty acid of milkfat, has two α-methylene groups and these are the points of attack in the free radical chain reaction. Hydrogen abstraction at C_8 and C_{11} results in the formation of two allylic radicals.

Oxygen attack at the end carbons of each radical produces an isomeric mixture of C_8-, C_9-, C_{10}-, and C_{11}-allylic hydroperoxides. It has been shown that these hydroperoxides are formed in about equal amounts (Frankel, 1962, 1979).

For linoleic acid and linolenic acid, polyene non-conjugated systems, the preferential points of attack are the α-methylene groups located between the double bonds. These α-methylene groups are doubly activated by the two adjacent double bonds, which makes them much more susceptible to oxidation than the α-methylene groups in fatty acids with one double bond. In linoleic acid, hydrogen abstraction at the C_{11} position along with oxygen attack produces an equal mixture of conjugated C_9- and C_{13}-diene hydroperoxides (Chan and Levett, 1977). The preferential formation of the free radicals at the C_9 and C_{13} positions is explained by the fact that these products could be resonance stabilized by a conjugated diene system. In linolenic acid, hydrogen abstraction at C_{11} and C_{14} results in the formation of a mixture of isomeric C_9-, C_{12}-, C_{13}- and C_{16}-hydroperoxides (Chan and Levett, 1977). In theory, six isomeric hydroperoxides are possible from linolenic acid oxidation, however, C_{11}- and C_{14}-hydroperoxides have not been found. This phenomenon is also explained by the theory of resonance stabilization.

Hydroperoxides are the primary products resulting from the autoxidation of unsaturated fatty acids. These hydroperoxides are unstable and readily decompose. In particular, the primary hydroperoxides of linolenate easily decompose to secondary dihydroperoxides because of the presence of active methylene groups in their structures. The main products of hydroperoxide decomposition are saturated and unsaturated aldehydes. Other products, such as unsaturated ketones, saturated and unsaturated alcohols, saturated and unsaturated hydrocarbons, and semi-aldehydes, have been observed in the decomposition of hydroperoxides of oxidized lipid systems. In addition to the major fatty acids, milk also contains many minor polyunsaturated acids, hence the oxidation of dairy products can lead to a multitude of saturated and unsaturated aldehydes (Parks, 1974).

Prevention of oxidation by packaging

Since reactive oxygen species are instrumental in initiating many of the oxidatively damaging reactions in food systems, it would be desirable to inhibit these reactions. For the long-term storage of foods, it often requires almost complete removal of headspace and dissolved oxygen. Some antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tocopherols probably can react with a variety of activated oxygen species including free radicals ($ROO^\cdot$, $RO^\cdot$) and singlet oxygen to minimize the oxidation of milkfat (Abbot and Waite, 1962; Hammond, 1970). However, addition of antioxidants in standard dairy products is not permitted, except for α-tocopherol which is less effective in retarding lipid oxidation compared to the synthetic phenolic antioxidants.

Alternatively, the shelf life of many food products has been greatly extended through improved packaging technology. One approach in food packaging is to store the food in an atmosphere as low in oxygen as possible to prevent or retard lipid deterioration. Taylor et al. (1979) recommended low temperatures coupled with oxygen barrier packaging or nitrogen flushing for the storage of dried foods. Warmbier and Wolf (1976) found that the oxygen levels can be reduced to 2% by flushing with inert gases. The use of nitrogen flushing or vacuum packaging has been found to be effective in minimizing the formation of undesirable flavor compounds in dry whole milk during storage (Tamsma et al., 1967,1973). Min et al.(1989) also demonstrated the beneficial effects of gas flushing with a mixture of 92% nitrogen and 8% hydrogen in retarding lipid oxidation in dry whole milk by measuring formation of volatile compounds in the headspace of packaging system. Samples packed in air were much more oxidized.

Although the oxidation of lipids may be controlled by gas flushing, residual oxygen may still cause deterioration in enclosed systems, especially for dry milk products. Mucha et al. (1961) noted that extremely low oxygen concentrations are necessary to prevent oxidative flavors in reconstituted foam spray-dried whole milk. Berlin and Pallansch (1963) reported

that it is possible to entrap oxygen inside dry milk particles during processing. In order to reduce the residual oxygen level in packaging systems to satisfy product requirements, it is necessary to obtain an efficient inert gas flush system and a scavenging system to tie up any oxygen which may be entrapped in the product or which may permeate through the packaging material (Berlin and Pallansch, 1963).

Many researchers have developed oxygen absorbers, also referred to as oxygen removers and oxygen scavengers, and evaluated the effect on food product quality and stability. A free oxygen absorber which consisted of iron powder, ferrous sulfate and a hygroscopic substance was developed by Maude et al. (1925). The first application of oxygen absorbers in preserving dry food quality was reported by Isherwood (1943). Later, a new oxygen scavenging system was devised by King et al. (1955). The system involved flushing the pack containing palladium pellets and product with a combination of hydrogen and nitrogen gas. Palladium catalyzed the reaction: $2H_2 + O_2 \rightarrow 2H_2O$ to remove residual oxygen from the headspace. Kuhn et al. (1970) demonstrated that a scavenger system containing polyvinyl alcohol as the principal gas barrier medium was very effective in achieving and maintaining very low residual oxygen concentrations in packages containing whole milk powder. In order to improve the reliability of the scavenging activity, Zimmerman et al. (1974) changed the film structure of the oxygen scavenger pouch to polyester/foil/ionomer/

catalyst/ionomer. This oxygen scavenging system significantly improved the flavor stability of whole milk powder during storage.

Another type of oxygen absorber consisting of dithionite (sodium hyposulfite and hydrosulfite), iron powder and organic reductions was developed by Fujishima (1977). It was claimed that sulfur dioxide (SO_2) was formed during the oxygen scavenging reaction and was trapped in the food product. This type of oxygen absorber has been applied successfully to the storage of vegetable oil, fried beans and dry instant noddles (Saito, 1979). In 1981, Rooney developed a new in-pack deoxygenation system, by using organic compounds immobilized in polymer media, for oxygen scavenging from headspaces in sealed packages. The results demonstrated that the oxygen content of plastic pouches containing air was reduced from 21 % to 1 % in 6 minutes. Recently, Sakamaki et al. (1988) conducted a study with oat cereal packaged in materials of different oxygen barrier properties with or without pouched-type oxygen absorbers, which consisted of a mixture of iron powder, catalysts and water. The absorbers were effective in delaying lipid oxidation in the oat cereal during storage.

Oxidation of Cholesterol

Description of cholesterol

Cholesterol (C_{27} H_{45} OH), cholest-5-en-3β-ol, is the major sterol in mammalian tissues. It is present in all

cellular membranes, especially the plasma membrane, and found in association with phospholipids, may control the passage of substances across such membranes (Dugan, 1987). By regulating fluidity of the membrane, cholesterol regulates membrane permeability, thereby exercising some control over what may pass into and out of the cell (Hunt and Groff, 1990). The total cholesterol content in all mammalian species is between 0.1% to 0.2% of the body weight of adults (Gibbons et al., 1982). Nervous tissue and the brain together contribute the largest proportion of the total body cholesterol.

Cholesterol is a non-polar simple lipid and contains a cyclopentanophenanthrene ring structure. The ring structure has an eight carbon side chain at the C_{17} position of the D ring, two methylated tertiary carbons at the C_{10} and C_{13} positions, a double bond at the C_5-C_6 position and a hydroxyl group at C_3. The main features of its stereochemistry are shown in Figure 1.

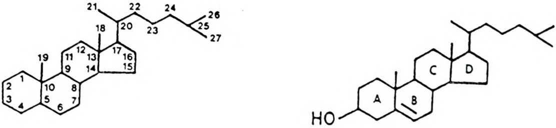


Figure 1. Structure of cholesterol

Mechanism of cholesterol oxidation

Being an unsaturated lipid, cholesterol readily undergoes oxidation in the presence of oxygen and light by a free radical process. Exposure of cholesterol to air, heat, light and irradiation may result in a variety of oxidation products. Free cholesterol is more stable towards oxidation than its esters (Korahani et al., 1982).

The major pathways of cholesterol oxidation have been elucidated and reviewed by Smith (1981) (Figure 2). He proposed two free radical mechanisms for cholesterol oxidation: (1) hydroperoxide formation in the B-ring and side-chain; and (2) formal dehydrogenation of the 3β-alcohol group.

$$RH + O_2 \text{ ------> } ROOH \quad \text{------------------} \quad (1)$$

$$RCH(OH) + O_2 \text{ ------> } RC{=}O + H_2O_2 \quad \text{-------} \quad (2)$$

In equation 1, the major mode of cholesterol oxidation, R mostly refers to C_7, C_{20} and C_{25}; moreover, it also refers to C_{24}, C_{26} or other carbons in the side chain. The C_7 position, the carbon adjacent to the double bond, is most sensitive to molecular attack by oxygen. The initial reaction involves the abstraction of the allylic C_7 hydrogen and reaction with ground state dioxygen ($^3O_2$) to form C_7 peroxy radicals. These peroxy radicals are stabilized by hydrogen abstraction to form the epimeric 7α- and 7β- hydroperoxides (Smith and Hill, 1972), which are the first detectable stable products. Both

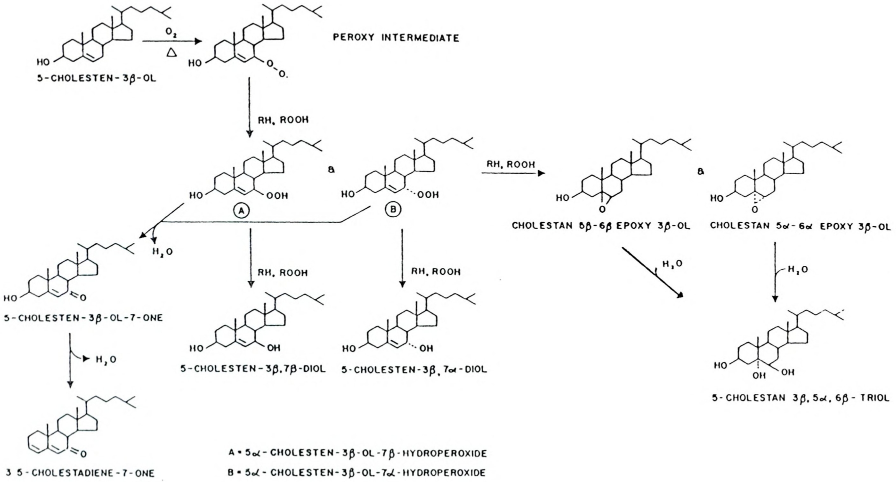


Figure 2. The major pathways of cholesterol oxidation (Luby, 1982)

hydroperoxides are subsequently decomposed to form therespective diols, or yield 7-ketocholesterol via direct dehydration (Van Lier and Smith, 1970; Smith et al., 1973). Epoxides, secondary oxidation products of cholesterol, are formed by the reaction of an unoxidized cholesterol molecule with 7-hydroperoxides. In terms of thermodynamic stability, the levels of 7β-diol and β-epoxide are predominant over those of the 7α-diol and α-epoxide, respectively, during the cholesterol oxidation process. Further hydration of these epoxides results in the formation of cholestan-3β,5,6β-triol (Smith and Kulig, 1975).

The presence of the tertiary C_{20} and C_{25} atoms in the side chain adds to the centers that are sensitive to oxidation. Oxidation reactions occurring at these positions tend to form relatively stable radicals via hydrogen abstraction by other free radicals (Van Lier and Smith, 1970, 1971). These radicals can then react with molecular oxygen to form hydroperoxides. These hydroperoxides then continue the chain propagation reactions, involving other cholesterol radicals to yield the corresponding hydroxycholesterols upon reduction or ketones via dehydration.

In the minor mode of cholesterol oxidation (equation 2), the 3β-alcohol group may undergo oxygen-dependent dehydrogenation involving initial C_3 radical formation and reaction with $^3O_2$ to form 3-hydroxy-3-peroxyl radicals. The 3-peroxyl radicals are then stabilized by elimination of the

elements of H_2O_2, resulting in the formation of cholest-5-en-3-one (Ansari and Smith, 1978).

Biological effects of cholesterol oxides

Cholesterol oxidation products have received considerable attention in recent years because of the biological activities associated with the etiology of certain human diseases. Based on studies of the biological effects and metabolic pathways, cholesterol oxidation products have been linked with carcinogenesis, cytotoxicity, atherogenesis, and inhibition of cholesterol synthesis (Smith, 1981; Addis and Park, 1989; Kumar and Singhal, 1991).

The atherosclerotic effects of diets containing cholesterol have well documented for many decades. However, the possible correlation of atherosclerosis and cholesterol oxides was not established until the reports of Imai et al. (1976). By feeding concentrated impurities from USP-grade cholesterol to rabbits, they observed an increase in frequency of dead or dying aortic smooth muscle cells (an index of angiotoxicity) and induced focal edema 24 hours after administration of the contaminant at a gavage of 250 mg/kg body weight. Administration of new and 5-year-old cholesterol produced similar effects, with the older sample exhibiting the greater atherosclerotic response. However, purified cholesterol showed no increase in degenerative cells. Imai and co-workers (1980) conducted a similar experiment to

further confirm the angiotoxicity of cholesterol oxides by injecting synthetic oxygenated sterols into rabbits. They concluded that oxygenated sterols, not cholesterol, may play a primary role in arterial wall injury and lesion development. More recent studies have also provided evidence that certain cholesterol oxides show atherogenic effects (Baranowski et al., 1982; Peng et al., 1982; Addis, 1986). A large number of cholesterol oxides have been evaluated for their atherogenicity. Addis (1986) concluded that the cholesterol oxides regarded as most atherogenic are cholestane-triol and 25-hydroxycholesterol.

The inhibitory effect of cholesterol oxides on cholesterol biosynthesis has also been thoroughly investigated during the past two decades. Kandutsch and Chen (1973, 1974) demonstrated that certain oxygenated sterols influenced cholesterol biosynthesis by inhibiting the activity of HMG-CoA reductase (3-hydroxy-3-methylglutaryl-CoA), a role-limiting enzyme in cholesterol biosynthesis. According to these reports, 25-hydroxycholesterol was the most potent inhibitor of cholesterol biosynthesis. However, Brown and Goldstein (1974) reported that the most potent inhibitor of cholesterol biosynthesis was probably 7-ketocholesterol, which was 100 times more potent than cholesterol. Parish et al. (1986) stated that the structural features of cholesterol oxides are related to their ability to repress the activity of HMG-CoA reductase. As a general trend, the inhibitory effect increases as the distance between the C_3 and the second oxygen function becomes greater.

Because of the important biological activities of cholesterol in cells and cell membranes, the cytotoxic effects of cholesterol oxidation products have also been the major focus of many studies. Higley and Taylor (1984) demonstrated that some cholesterol oxides can cause inhibition of cell growth and cell death on cultured medial smooth muscle cells. Addition of cholestan-triol and 25-hydroxycholesterol to the cultured smooth muscle cells results in cell death, whereas purified cholesterol showed no cytotoxicity (Peng et al., 1979, 1982). The inhibitory effect of cholesterol oxides on the activities of the enzyme Na^+, K^+ ATPase and 5'-nucleotidase has also been observed (Peng et al, 1985; Peng and Morin, 1987).

Another risk associated with the intake of cholesterol oxides is the suspected carcinogenicity of α-epoxide. Black and Douglas (1972, 1973) observed the formation of α-epoxide in the skin of human and hairless mice after exposure to UV radiation. Chan and Black (1974) also showed that increases of the α-epoxide level in the skin of hairless mice subjected to UV light coincided with a rapid increase in the number of tumor incidences. Although there is no direct evidence to link α-epoxide to development of cancer, the presence of epoxycholesterol may still play an indirect toxicological role.

Determination of cholesterol oxidation products in foods

During the past decade, various methods have been developed for the identification and quantification of cholesterol oxidation products in foods. These methods include thin-layer chromatography (TLC), packed column gas chromatography (GC), capillary column GC, and high-performance liquid chromatography (HPLC).

Although TLC remains one of the best methods for rapid analyses of cholesterol oxides (Smith, 1987), a few compounds, e.g., 7-ketocholesterol and 5,6-epoxides, are poorly resolved by TLC (Maerker, 1987). HPLC has been successfully used for identification and quantitation of some cholesterol oxides. The procedure is highly effective for the separation of diols and hydroperoxides, but triol is often not determined by adsorption HPLC (Maerker, 1987). Many workers have demonstrated that satisfactory resolution of the major oxidation products of cholesterol can be achieved by capillary GC [(Gumulka et al., 1982; Missler et al., 1985; Park and Addis, 1985b, 1986; Maerker and Unruh, 1986; Nourooz-Zadeh and Appelqvist, 1988 a,b; Sander et al., 1988, 1989; Morgan and Armstrong, 1989; Engeseth, 1990; Pie et al., 1991)]. Smith (1987) reviewed the analytical methods for oxysterols and concluded that GC analysis of the oxysterol trimethylsilyl ethers on capillary columns with suitable bonded or liquid phases is the most powerful technique at the present time.

The quantitative determination of cholesterol oxides in

foods is prone to several potential errors. One is the generation of artifacts during the analytical procedure. Another is the breakdown of oxides during analysis (Smith, 1981). It is especially important to observe the formation of 5α-cholestane-3β,5,6β-triol which is a spontaneous breakdown product of the two epoxy compounds. Another error arises from the breakdown of cholesterol oxides during saponification (Park and Addis, 1985).

The presence of large quantities of cholesterol in foods interferes with the quantification of some cholesterol oxidation products. Therefore, cholesterol must be removed prior to analysis. Previous workers have frequently employed hot alkaline saponification to hydrolyze lipids and to concentrate non-saponifiable sterols (Flanagan et al., 1975; Ryan et al., 1981; Finocchiaro et al., 1984). Several reports, however, have demonstrated that some cholesterol oxides suffered from structural alterations under such harsh treatment (Tsai and Hudson, 1981; Maerker and Unruh, 1986; Park and Addis, 1986). For example, the isomeric epoxides and 7-ketone can decompose to various degrees during this procedure, thereby generating the triol and the diene, respectively. There are many literature reports detailing losses of single cholesterol oxides. Chicoye et al. (1968) reported that when 7-ketocholesterol was heated with a potassium hydroxide solution , cholesta-3,5-dien-7-one was the major degradation product produced. Tsai et al. (1980)

reported a loss of approximately 75% of the α-epoxide after saponification, due to the hydrolysis of the epoxide ring. Maerker and Unruh (1986) reported no losses of the isomeric epoxides, but a substantial loss of 7-ketocholesterol during hot saponification. Moreover, they also demonstrated that 6-ketocholestanol, a compound commonly used as an internal standard, is affected by base and is partially destroyed by hot alkali as well. Therefore, it is necessary to develop an alternative technique for more accurate quantitation of cholesterol oxides.

Park and Addis (1985a) developed a mild method, avoiding saponification, for the analysis of C_7 cholesterol oxides. Total lipid extracts were fractionated on silica gel columns to concentrate trace sterol oxides from triacylglycerols, cholesterol, and phospholipids. These results showed that the combination of silica gel column chromatography and HPLC with UV detection was quite reliable and reproducible. Later, they prepared cholesterol and its oxidized derivatives through cold saponification and found that no detectable amounts of artifactual oxidation products were produced during the sample preparation. Nourooz-Zadeh and Appelqvist (1987) also demonstrated that the breakdown of cholesterol oxides was minimized by the elimination of the saponification step. Therefore, Smith (1987) has emphasized that some of the quantitative data of cholesterol oxides must be viewed with skepticism because of the insidious nature of cholesterol

oxidation.

Cholesterol Oxidation Products in Foods

Food products of animal origin are often subjected to various processing treatments which may induce the oxidation of cholesterol. Until recently, however, the deterioration of foods due to the oxidation of cholesterol was seldom examined. Because cholesterol oxides are present in foods in rather low concentration, their isolation and determination presented challenging analytical problems. In the past decade, as a result of the development of appropriate analytical methods, cholesterol oxides have been found in a variety of foodstuffs such as egg products (Tsai and Hudson, 1984; Missler et al., 1985; Sugino et al., 1986; Nourooz-Zadeh and Appelqvist, 1987; Morgan and Armstrong, 1989; Sander et al., 1989), dairy products (Finocchiaro and Richardson, 1983; Luby et al., 1986; Nourooz-Zadeh and Appelqvist, 1988 a,b; Sander et al., 1988; Sander et al., 1989), heated fats (Ryan et al., 1981; Bascoul et al., 1986; Park and Addis, 1986 a,b), meat products (Park and Addis, 1985 a; Higley et al., 1986; Park and Addis, 1987; Engeseth and Gray, 1989; Zubillaga and Maerker, 1991; Pie et al., 1991), and other deep-fried foods (Lee et al, 1985; Zhang et al., 1991). Some of the cholesterol oxidation products isolated from foods are listed in Table 3.

Formation of cholesterol oxidation products in foods may occur from oxidation before, during, or after processing.

Table 3. Cholesterol oxidation products in foods [a]

Food	Oxysterols found (μg/g)
<u>Egg Products</u>:	
Dried egg yolk	3β,7α-diol (50)
	3β,7β-diol (25)
	3β,25-diol (25)
	cholest-5-ene-3β,4β-diol
	7-ketone (22)[b]
	5α,6α-epoxide (79)[b]
	5β,6β-epoxide (28)[b]
Spray-dried egg	3β,7α-diol (2.2-20.2)
	3β,7β-diol (1.6-24.6)
	5,6-epoxides (1.6-24.9)
	7-ketone
	5α,6α-epoxide (17.4)
	5β,6β-epoxide (31.8)
	triol (0-1.2)
<u>Milk Products</u>:	
Whole milk powder[c]	3β,7α-diol (0.3-1.0)
	3β,7β-diol (0.7-1.5)
	5α,6α-epoxide (0.5-2.5)
	5β,6β-epoxide (1.1-3.2)
	7-ketone (5.1-9.2)
Cheeses	3β,7α-diol (0.1-0.8, 3-6)
	3β,7β-diol (0.2-0.9, 3-6)
	5,6-epoxides (0.1-0.9, 6-32)
	triol
Butteroil	3β,7α-diol (0.1-0.4, 20-60)
	3β,7β-diol (0.3-1.2, 30-90)
	7-ketone
	cholesta-3,5-dien-7-one
	5,6-epoxides (0.1-0.2, 20-30)
	triol (0.1)
	3β,25-diol (0.17)
<u>Meat Products</u>:	
Heated beef tallow	3β,7-diols
	7-ketone
	cholesta-3,5-dien-7-one
	5,6-epoxides
	triol
Minced pork (raw, cooked)[d]	3β,7α-diol (0.19, 0.64)
	3β,7β-diol (0.28, 0.85)
	5α,6α-epoxide (0.22, 0.39)
	5β,6β-epoxide (0.35, 1.01)
	7-ketone (0.92, 2.25)
	3β,20-diol (0, 0.14)
	3β,25-diol (0.13, 0.38)
	triol (0.04, 0.06)

Raw chicken muscle[e]	5α,6α-epoxide (0.066) 5β,6β-epoxide (0.058) 7-ketone (0.129)
Other Products:	
French fried potatoes[f]	3β,7-diols 5,6-epoxides 7-ketone 3β,25-diol triol

[a] Adapted from Smith (1987)
[b] From Sander et al. (1989)
[c] From Nourooz-Zadeh and Appelqvist (1988)
[d] From Pie et al. (1991)
[e] From Zubillaga and Maerker (1991)
[f] From Zhang et al. (1991)

Smith (1981) reported that the major oxidation products of cholesterol in foodstuffs include 25-hydroxycholesterol, cholestane-triol, 7α-hydroxycholesterol, 7β-hydroxycholesterol, 7-ketocholesterol, α-epoxide, β-epoxide, and cholesta-3,5-dien-7-one. Heating foodstuffs in air or placing foodstuffs under illumination can increase the levels of cholesterol oxides. Missler et al. (1985) reported that the spray-drying of eggs using direct heat (gas-fired) leads to increased cholesterol oxide concentrations in comparison to the use of indirect heat (steam).

Cholesterol Oxidation Products in Whole Milk Powder

Cholesterol is a minor but essential component of all mammalian milks (Hilditch and Williams, 1964; Sabine, 1977). The amount of cholesterol in milk varies among species and tends to vary proportionally to the triacylglycerol concentration in different species (Moore and Richardson, 1965; Sabin, 1977). The cholesterol content of cow's milk is small, 0.20 %-0.4 % of the total lipids (Nataf et al., 1944; Moore and Richardson, 1965; Brink, 1968; Sweeney and Weihrauch, 1977) and varies with the seasons (Nieman and Groot, 1950).

Cholesterol in milkfat is esterified or unesterified (Nataf et al., 1948). However, most cholesterol (85 to 90%) in milk exists as free cholesterol (Patton and McCarthy, 1963). The bulk of this cholesterol is associated with the phospholipids in the formation of the surface membrane of fat droplets. It is derived from the plasma membrane of the secretory cells of the mammary gland. A minor portion of the cholesterol exists in an esterified form, usually combined with long-chain fatty acids. There are many investigations of the properties of the fatty acid in cow's milk cholesteryl esters (Patton and McCarthy, 1963; Keenan and Patton, 1970; Parks, 1980; Wood and Bitman, 1986). Recently, Wood and Bitman (1986) found that in the cholesteryl esters in the milk of mature cows, 62 % of the fatty acids were saturated. Linoleic acid represented 27.1% of the fatty acids present.

As dried milk powders contain cholesterol, it has been suggested that they may have an adverse impact on human health. Wilson (1976) considered that the consumption of old powdered whole milk may be a significant risk factor in human atherosclerosis. Taylor et al. (1979) reported that dried powdered foods of animal origin stored in air at room temperature may be major sources of angiotoxic sterol oxidation products in the diet. Leduc (1980), as cited by (Luby, 1982), also demonstrated that ingestion of dehydrated milk powder caused a high incidence of tumors in mice.

Dried milk products are processed under various thermal conditions and are stored at ambient temperature for prolonged periods. These treatments provide ample opportunities for the oxidation of cholesterol to occur. However, much of the research regarding cholesterol oxidation in dairy products has focused on butter and cheese. Although there are reports on the occurrence of cholesterol oxides in milk powder products (Flanagan et al., 1975; Finocchiaro and Richardson, 1983), no quantitative data are available on the levels of these oxides in milk powder.

Nourooz-Zadeh and Appelqvist (1988a) reported the concentrations of cholesterol oxides in milk powder products (cream, whole milk and skim milk powder) as related to processing technology and storage. Their results revealed that all freshly-processed milk powder products contained less than 0.1 mg cholesterol oxides/ kg total lipids for low and medium heat powders. On the other hand, freshly spray-dried whole milk and skim milk powders from "high heat" conditions had quantifiable amounts of some important cholesterol oxides, e.g., cholest-5-ene-3β,7α-diol, cholest-5-ene-3β,7β-diol, 5,6α-epoxy-5α-cholestan-3β-ol,5,6β-epoxy-5β-cholestan-3β-ol,

and 3β-hydroxycholest-5-en-7-one. The epimeric 7-hydroxycholesterols and 7-ketocholesterol were the major oxidation products, followed by the isomeric 5,6-epoxycholesterols. Furthermore, one-year-old spray-dried whole milk powder samples exhibited increased levels of cholesterol oxides. The quantitative pattern for the concentration of the oxidation products of cholesterol in milk powder products was almost similar to that observed in the analysis of dehydrated egg yolk and egg yolk mix products (Nourooz-Zadeh and Appelqvist, 1987). However, 7-ketocholesterol was found in relatively higher concentrations in milk powder products than in egg yolk powders.

MATERIALS AND METHODS

Materials

Cholesterol oxide standards

Cholesterol and 5α-cholestan-3β-ol-6-one (6-ketocholesterol) were purchased from Sigma Chemical Co. (St. Louis, MO). The following cholesterol oxide standards were purchased from Steraloids Inc., Wilton, NH: 5α-cholestane, cholestan-5,6α-epoxy-3β-ol (α-epoxide), cholestan-5,6β-epoxy-3β-ol (β-epoxide), 5-cholesten-3β-ol-7-one (7-ketocholesterol), cholestan-3β,5α,6β-triol, 5-cholesten-3β,20α-diol (20α-hydroxycholesterol), 5-cholesten-3β,25-diol (25-hydroxycholesterol), 5-cholesten-3β,7α-diol (7α-hydroxycholesterol) and 5-cholesten-3β,7β-diol (7β-hydroxycholesterol).

Fatty acid standards

The methyl esters of butyric acid, stearic acid, oleic acid, linoleic acid, and linolenic acid were purchased from Sigma Chemical Co. (St. Louis, MO). Standard reference mixtures of fatty acid methyl esters (C_6, C_8-C_{16}) were also obtained from Supelco, Inc. (Bellefonte, PA).

Packaging materials

The pouched oxygen absorbers (consisting of iron powder, water and catalysts) were obtained from Multiform Desiccants, Inc., Buffalo, NY. Polyethylene pouches (Ziploc freezer bags, 5"x7", 2 mil thickness) were purchased from a local supermarket. Crimp seal glass vials (100 ml), teflon septa, and aluminum crimp-on caps were obtained from Supelco, Inc. (Bellefonte, PA).

Reagents

Pyridine and bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) were obtained from Pierce Chemical Co. (Rockford, IL). All other reagents used in the experiments were of analytical grade.

Preparation of Whole Milk Powders

Liquid whole milk was supplied by a local producer in County Cork, Ireland. A pilot-scale Anhydro Lab3 spray drier with pneumatic nozzle atomization was used to spray dry the milk at the National Dairy Products Research Centre (Moorepark, Fermoy, Co. Cork, Ireland). The drier was equipped with electrical heating elements for indirect air heating. It also had the capability of being converted to direct gas-fired heating by attaching a gas burner to the extended air inlet duct. Whole milk powder samples were manufactured by direct low-NO_x gas-fired heating and direct high-NO_x gas-fired heating of the drier, respectively, with the fitting of a CXA gas burner (digram shown in appendix 1) and a standard gas burner (Radiant Superjet model GX 25) to the drier. Samples of whole milk powder produced by the indirect (electric) air heating system were used as experimental controls.

Whole milk powder samples were vacuum packaged on the same day as drying and air freighted to Michigan. On receiving at MSU, the whole milk powder samples were refrigerated at 4°C for one week until required for analysis. After the initial analyses (TBA test, color measurement, total lipid and total cholesterol analysis), duplicate samples (60 g) produced by all drying methods were placed into polyethylene pouches (5" x 7", 2 mil thickness), crimp seal glass vials (100 ml) with oxygen absorbers, and crimp seal glass vials without oxygen absorbers, respectively. After filling, the sample pouches were heat sealed and the sample vials were sealed by aluminum crimp-on caps with teflon septum. The packed samples were stored in the dark for six months at 20°C and 40°C. The whole experiment was repeated twice.

Methods

Measurement of lipid oxidation

Lipid oxidation in the stored powder samples was measured using the thiobarbituric acid (TBA) procedure. Ten grams of

the whole milk powder were mixed with 97.5 ml of deionized water and added to a distillation flask containing silicone emulsion antifoam (Thomas Co., Swedesboro, NJ), glass beads, and 2.5 ml of hydrochloric acid ($H_2O/HCl= 2:1$, v/v). By using the distillation method of Tarladgis et al. (1964), 50 ml of distillate were collected. A 5 ml aliquot of the distillate was added to a test tube containing 5 ml of aqueous TBA solution (Crackel et al., 1988). The capped tubes were lightly vortexed, and then heated in a water bath at 100°C for 35 minutes. The tubes were cooled to room temperature and the color intensity quantitated by measuring the absorbance at 532 nm using a double beam Bausch and Lomb Spectronic 2000 spectrophotometer (Rochester, N.Y.).

Lipid extraction

Total lipids were extracted from the milk powders using a slightly modified version of the method of Folch et al. (1957). Whole milk powder (1 g) was homogenized using a Ultra-Turrax type of homogenizer (Tekmar Co., Cinn., OH) in a 150 ml beaker for 1 min with 15 ml methanol, then chloroform (30 ml) was added and the process continued for an additional 2 min. The homogenate was filtered through Whatman filter paper (No.1) to remove whole milk powder particles, which were then washed with 30 ml chloroform/methanol (2:1, v/v). After washing, the combined filtrates were added to 10 ml deionized water and shaken by hand for thirty seconds to ensure mixing.

The mixture was then transferred to a 100 ml centrifuge tube and centrifuged (1000 x g) for 10 min. The upper aqueous layer was removed by aspiration. The inside wall of the centrifuge tube was further rinsed with 3 x 1.5 ml aliquots of a solvent mixture of chloroform/methanol/water (3:48:47, by volume). The lower cholroform phase was collected, dried over anhydrous Na_2SO_4, and evaporated to dryness using a rotorary evaporator. The lipid extract was redissolved in 5 ml hexane and stored at -20°C until required for further analysis.

Total cholesterol determination

The total cholesterol content of the whole milk powders was determined by the method of Adams et al. (1986). Milk powder (3-5 g) was accurately weighed into a 250 ml flat bottom refluxing flask to which was added 8 ml 50 % KOH and 40 ml alcohol. This mixture was saponified by refluxing and stirring for one hour. After the addition of 60 ml alcohol, the mixture was allowed to cool down and toluene (100 ml) was added and vigorously stirred for one min. The mixture was extracted in a 500 ml separatory funnel with 110 ml 1N KOH, shaking vigorously for 30 sec and then the lower aqueous layer was discarded upon separation. To the toluene layer, 40 ml 0.5N KOH were added and the separatory funnel was rotated gently. Again the lower layer was discarded and the toluene extract was washed five times with water (100 ml). The toluene extract was dried over anhydrous Na_2SO_4. A 50 ml

aliquot was removed and rotary evaporated to dryness, washed with acetone, dried, and redissolved in 3 ml dimethylformamide.

Gas chromatographic (GC) analysis was carried out using a packed (1% SE-30 on 100/120 Gas Chrom Q) column and a Hewlett Packard 5840A gas chromatograph (Avondale, PA) under isothermal conditions (230°C for 20 min). The injector and detector temperatures were held at 275°C and 300°C, respectively. Helium with a flow rate of 25 ml/min was used as the carrier gas.

Cholesterol oxide determination

The procedures used to concentrate and derivatize the cholesterol oxides were slightly modified from the method of Morgan and Armstrong (1989). The lipid extract (250 mg) with 15 μg of internal standard (6-ketocholesterol) was transferred to an Supelclean LC-Si SPE tube (Supelco, Bellefonte, PA) which had been prewashed with 5 ml hexane. The Supelclean LC-Si SPE tube was then washed with 10 ml hexane and 15 ml hexane/diethyl ether (95:5, v/v) to elute most of the triacylglycerols from the column. The remaining triacylglycerols and cholesterol were eluted by washing the column with 25 ml hexane/diethyl ether (90:10, v/v) and 15 ml of hexane/diethyl ether (80:20, v/v). The cholesterol oxides were eluted off the column with 10 ml acetone. The acetone fraction was evaporated to dryness under nitrogen and

redissolved in 50 μl pyridine plus 50 μl BSTFA in a 1 ml vial. This mixture was placed in the dark at room temperature for 1 hr to form the trimethylsilyl ether derivatives of the cholesterol oxides.

GC analysis of the cholesterol oxide derivatives was performed using a Hewlett Packard 5890A gas chromatograph (Avondale, PA) equipped with a flame ionization detector. The cholesterol oxide derivatives were separated on a DB-1 capillary column (15 m x 0.25 mm i.d., 0.1 μm film thickness; J&W Scientific Inc., Ann Arbor, MI). Helium was used as carrier gas at a head pressure of 50 psi and with a flow rate of 27 ml/min. The oven temperature was programmed from 170°C to 220°C at a rate of 10°C per min, then increased to 234°C at a rate of 0.4°C per minute, held for 5 minutes, and then increased to 256°C at a rate of 2°C. The temperatures of the injection and detector ports were 270°C and 300°C, respectively.

Fatty acid composition of whole milk lipids

The determination of the fatty acid profiles of milkfat was performed by the method of Badings and Jong (1983). Whole milk powder lipids (100 mg) were dissolved in 6 ml pentane in a screw capped test tube, to which was added 0.06 ml 2M sodium methoxide solution. The contents of the capped tube were stirred vigorously for 1 min at room temperature on a vortex mixer. The sediment of sodium glycetolate was separated by

centrifugation at 1000 x g for 3 min. One μl sample of the clear supernatant was then taken for GC analysis.

Fatty acid analyses were conducted on a HP 5890A system with a DB-225 capillary column (30 m x 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific Inc., Ann Arbor, MI) and a flame ionization detector. An initial oven temperature of 40°C was held for 5 min, then programmed to 200°C at a rate of 10°C per minute and held for 20 min. Injector and detector temperatures were 275 °C and 300 °C, respectively. The GC was run in the split mode with a split ratio of 16. Peaks were identified by comparison of retention times with standard reference mixtures of fatty acid methyl esters.

Nitrite and nitrate analyses

Extraction of nitrite and nitrate from whole milk powder was achieved using the method of the International Dairy Federation Standard 95 (1980). A sample of whole milk powder (10 g) was dissolved in 156 ml warm deionized water (50-55°C). Then 12 ml 53.5 % (w/v) zinc sulfate solution, 12 ml 17.2 % (w/v) potassium hexacyanoferrate solution and 20 ml ammonium chloride buffer solution (pH 9.6-9.7) were added in the order described and mixed thoroughly using a vortex mixer after each addition. After 15 min, the mixture was filtered through Whatman filter paper (No.1) and the filtrate was collected.

Nitrate is quantitatively reduced to nitrite by passing the filtrate through a copperized cadmium column. Nitrite

analyses were performed using a Lachat Quikchem Automated Flow Injection Ion Analyzer (Mequon, WI) in which nitrite was diazotized with sulfanilamide, and then coupled with N-(1-naphthyl)ethylenediamine dihydrochloride to produce a water-soluble magenta dye. Absorbance was measured at 520 nm.

The nitrate content was calculated from the difference between the total nitrite (reduced nitrate plus original nitrite) content and the original nitrite content.

Color measurement

Color differences in the whole milk powder samples, as affected by storage and packaging, were determined using a Hunterlab ColorQUEST $45^o/0^o$ spectrophotometer (Hunter Assoc. Lab. Inc., Reston, VA). A standardized white tile (X=81.73, Y=86.51, Z=92.74) was used as a color reference.

Headspace oxygen measurement

The headspace oxygen contents of the packed samples were determined using a Carle 2153-B gas chromatography (Carle Inc., Anaheim, CA) with a molecular sieve 5A column (60/80 mesh, 8 m) and a column (50/80 mesh, 8 m) consisting of 20 % PPQ (Porapak Q) and 80 % PPN (Porapak N). A 500 ml sample of gas was withdraw from the package by a gas-tight syringe and injected into the sample loop of the GC. Helium was used as the carrier gas at a head pressure of 40 psi. The oven temperature was maintained at 50°C.

Statistical analysis

The experiment was conducted as a four factor (drying method x packaging x temperature x time) split-plot design with two replications. Statistical analysis of the data for cholesterol oxides, TBARS values, and color differences was performed using a Bonferroni t-test to analyze specific contrasts among temperature treatments and Duncan's multiple comparisons test for drying methods and packaging systems (Ott, 1988). The analysis of variances were performed using MSTAT-C microcomputer statistical program (Michigan State University, East Lansing, MI, 1989).

RESULTS AND DISCUSSION

Nitrite and Nitrate Concentrations in Whole Milk Powders

Oxides of nitrogen (NO_x), generated by the spray drying process, may serve as initiators of lipid oxidation in whole milk powders. To evaluate the efficiency of different spray drying techniques in reducing the NO_x level in the drying air, the concentrations of NO_x were measured using Draegar tubes during the spray drying process (Kelly, personal communication). In addition, nitrite and nitrate contents, a reflection of NO_x levels present in the spray drying chamber, in whole milk powders were also determined.

By using the low NO_x gas burner, the concentrations of NO_x in the flue gases at the burner throat were between 1.0 and 1.65 ppm. Following dilution of the flue gases by the drying air, the levels of NO_x were in the range 0.13 to 0.23 ppm. For the high NO_x burner, 8 ppm nitrogen oxides were detected in the air at the inlet of the dryer.

The average concentration of nitrite (NO_2^-) in direct high NO_x gas-fired whole milk powders was 0.11 μg/g. There was no detectable nitrite in samples prepared by the direct low NO_x and electrically heated processes. These data are not in agreement with the report of Kelly et al. (1989), who observed nitrite values of 0.81 and 0.59 μg/g for direct low NO_x gas-fired and electric heated samples of skim milk

powders, respectively.

The mean nitrate (NO_3^-) values were 5.33, 5.50 and 7.41 μg/g for electric heating, direct low NO_x and direct high NO_x gas-fired heating whole milk powders, respectively. The nitrate content in powders processed by the direct high NO_x gas-fired dryer was significantly ($P < 0.05$) higher than those of samples processed by direct low NO_x gas-fired and indirect electric heating. There was no significant difference in the nitrate contents of the direct low NO_x and electrically heated samples. These results were generally consistent with the report of Kelly et al. (1989), however, their data were approximately two-fold higher than those presented here.

Knipschildt (1986) noted that NO_x in the drying air are not only created from the air by thermal processes, but can also originate from nitrogen in the fuel during the spray drying process. Kelly et al. (1989) also demonstrated that background NO_x levels in the ambient air affected the flue gas NO_x levels and that the lowest values of NO_x were obtained during the winter period. Therefore, the variations in NO_x contents in spray-dried milk powders could be due to the different NO_x levels in the ambient air, and to the fluctuation of nitrogen content in the natural gas used during the combustion process.

In this study, it was apparent that the NO_x in whole milk powders could be effectively reduced using a direct low NO_x drying process. Based on these results, it was anticipated that the oxidative stability of lipids in whole milk powders would also be improved by using low NO_x drying processes, based on the fact that NO_x are known initiators of lipid oxidation.

Headspace Oxygen Content as Influenced by Packaging System

To examine the scavenging ability of oxygen absorbers and the influence of oxygen on the oxidative stability of whole milk powders, headspace oxygen contents of packaged samples were measured during the storage period. The oxygen concentrations in the headspaces of the PE pouches, glass vials without oxygen absorbers, and glass vials with oxygen absorbers were 20.1 %, 20.2 % and 0.03 %, respectively, 3 hours after packaging (Table 4). The headspace oxygen contents of whole milk powders packaged in PE pouches were approximately equal to the percent oxygen in air and remained constant during the storage period. This was due to the oxygen permeability of the polyethylene film which allowed oxygen to continuously penetrate through the packaging material from the atmosphere. However, the oxygen contents in the headspace of whole milk powders packaged in glass vials without oxygen absorbers decreased with storage time, with the samples stored at 40°C showing the greater decreases. After 6 months storage at 20°C and 40°C, the oxygen contents in the headspaces were 18.8 % and 6.8 %, respectively. It is

Table 4 - Headspace oxygen contents of whole milk powders packed in various packaging systems and held at 20°C and 40°C for 6 months.

Storage condition	Packaging system*	Headspace oxygen (%)	
		0 mo	6 mo
20°C	PE	20.1±0.3	20.1±0.8
	w/out OA	20.2±0.4	18.8±1.0
	w/ OA	0.03±0.01	0.24±0.07
40°C	PE	20.1±0.3	20.2±0.5
	w/out OA	20.2±0.4	6.8±2.5
	w/ OA	0.03±0.01	0.24±0.07

*PE - polyethylene pouch; w/out OA - glass vial without oxygen absorber; w/ OA - glass vial with oxygen absorber.

postulated that the oxygen in the headspace reacted with the whole milk powders and the reactive rate was accelerated by the higher temperature. On the other hand, oxygen absorbers reduced headspace oxygen in the glass vials to almost zero percent within 3 days. The headspace oxygen content increased to 0.24 % after 6 months of storage. This could be attributed to a small leakage in the seals of the vials.

Lipid Oxidation in Whole Milk Powders during Storage

Lipid oxidation is a major problem associated with the long-term storage of whole milk powders (Coulter et al., 1951; Lea et al., 1953; Shipstead and Tarassuk, 1953; Boon et al.,

1976; Tuohy, 1987). Many methods have been used for the measurement of lipid oxidation in such powders, including the 2-thiobarbituric acid test, peroxide value test, carbonyl measurement, oxygen absorption and sensory assessment. According to a comprehensive study by Tuohy (1987), each of the methods has its limitation. However, he reported that the TBA-reactive compounds (TBARS) value was a better index of milkfat oxidation than the peroxide value, oxygen absorption, or sensory assessment procedures. Tuohy (1987) also reported a reasonable correlation between the TBARS value and flavor acceptability of whole milk powder. Furthermore, the development of TBARS in whole milk powders during storage tended to precede an increase in the content of total carbonyl compounds, which are believed to be responsible for the off-flavors associated with oxidized milkfat (Boon, 1976). Therefore, it was concluded that the TBARS value is a more sensitive index of oxidative changes in whole milk powder than the total carbonyl content.

Based on the results reported by Tuohy (1987), only the TBARS value was used as a measure of lipid oxidation in this study. TBA-reactive compounds were isolated from whole milk powder by the steam distillation method of Tarladgis et al. (1964).

Effect of storage conditions on lipid stability in whole milk powders

The initial TBARS values of whole milk powder samples produced by indirect electric heating, direct low NO_x gas-fired and direct high NO_x gas-fired heating were 0.11, 0.12 and 0.13 (obtained by multiplying absorbance at 532 nm by 6.2, a factor determined by Crackel et al., 1988), respectively. TBARS development at the initial stages of lipid oxidation in the powders was not significantly influenced ($P < 0.05$) by the drying procedure. Steen (1977) reported TBARS values in the range of 0.020 to 0.042 (expressed as absorbance at 530 nm) in commercially produced whole milk powders immediately after manufacture. These results generally agree with the TBARS values reported here, even though the procedure used by Steen (1977) was different from that used in this study. Using a different TBA procedure, Mettler (1973) also indicated that a TBARS value of 0.008 (absorbance at 530 nm) was representative of freshly produced whole milk powder. Ward (1985) concluded that the TBA assay was both operator- and method-dependent. Therefore, comparison of TBARS results from different laboratories must be undertaken with caution.

The change in TBARS values of the milk powders with storage time was very distinct. During storage, the TBARS values increased in a linear manner for the samples packaged in PE pouches and glass vials without oxygen absorbers. A highly significant correlation was found between TBARS value and storage time. When TBARS values were plotted against storage time, correlation coefficients of 0.87 to 0.96 were obtained indicating a strong linear relationship. The best-fit regression lines of TBARS value versus storage time at 20°C and 40°C for the samples manufactured by different drying methods are shown in Table 5. However, a poor correlation (r= 0.55) between TBARS value and storage time was found in the samples packaged in glass vials with oxygen absorbers.

The damaging effect of temperature on the oxidative stability of whole milk powders packaged in PE pouches and in glass vials without oxygen absorbers was reflected in the development of TBARS, as illustrated in Figures 3 and 4. As expected, TBARS values of the powders stored at 40°C increased more rapidly than those of samples stored at 20°C. The means were significantly different ($P < 0.01$). However, the higher temperature did not influence TBARS development in powders packaged in glass vials with oxygen absorbers (Figure 5). After 6 months storage, the TBARS values of powders manufactured by direct high NO_x gas-fired heating, packaged in PE pouches, and stored at 20°C and 40°C were 0.45 and 1.41, respectively. When the same powders were packaged in glass vials without oxygen absorbers, similar results were observed. On the other hand, there was only a slight decrease in TBARS values when the samples were packaged in glass vials with oxygen absorbers and stored at 40°C, compared to samples stored at 20°C.

Table 5 - The relationship between storage time and TBARS values of whole milk powders processed by different drying methods, packaged in various packaging systems and stored for 6 months at 20°C and 40°C.

Storage condition	Packaging system*	Drying method	Best fit regression line**	r
20°C	PE	electric	$Y=0.10+0.03X$	0.867
		low-NO_x	$Y=0.11+0.04X$	0.927
		high-NO_x	$Y=0.13+0.05X$	0.909
	w/out OA	electric	$Y=0.12+0.04X$	0.961
		low-NO_x	$Y=0.12+0.05X$	0.941
		high-NO_x	$Y=0.14+0.06X$	0.882
	w/ OA	electric	$Y=0.11+0.004X$	0.276
		low-NO_x	$Y=0.10+0.02X$	0.701
		high-NO_x	$Y=0.12+0.02X$	0.762
40°C	PE	electric	$Y=0.07+0.12X$	0.958
		low-NO_x	$Y=0.24+0.20X$	0.938
		high-NO_x	$Y=0.09+0.21X$	0.885
	w/out OA	electric	$Y=0.11+0.08X$	0.942
		low-NO_x	$Y=0.14+0.11X$	0.916
		high-NO_x	$Y=0.16+0.14X$	0.926
	w/ OA	electric	$Y=0.10+0.01X$	0.493
		low-NO_x	$Y=0.11+0.008X$	0.560
		high-NO_x	$Y=0.13+0.007X$	0.500

*PE - polyethylene pouch; w/out OA - glass vial without oxygen absorber; w/ OA - glass vial with oxygen absorber.
**X= storage time (months), Y= TBARS value.

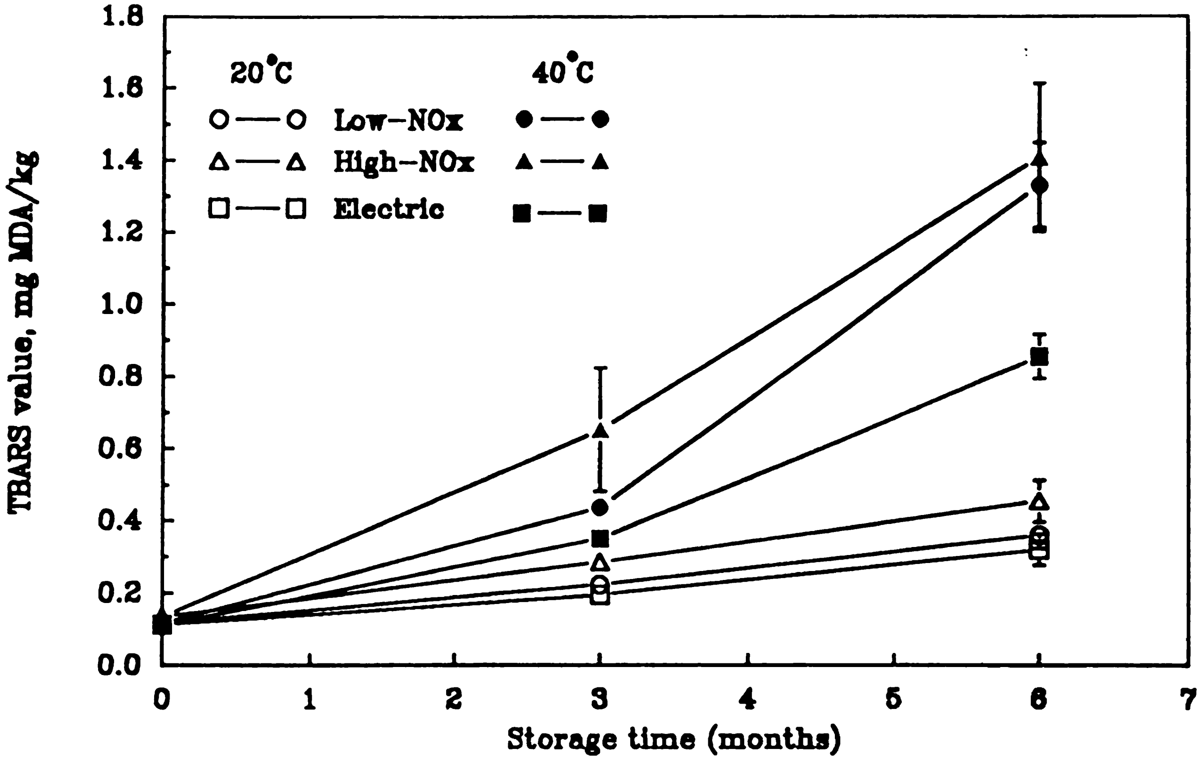


Figure 3. Development of TBARS in whole milk powders in PE pouches during storage as influenced by method of drying and storage temperature

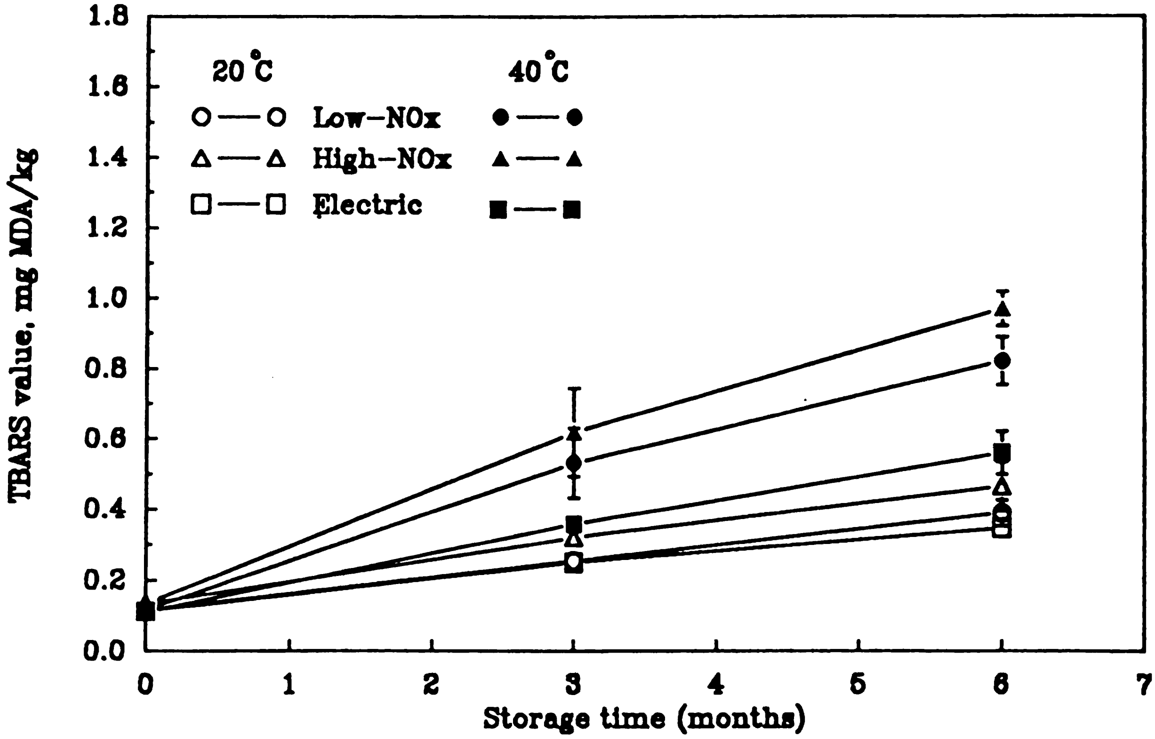


Figure 4. Development of TBARS in whole milk powders in glass vials without oxygen absorbers during storage as influenced by method of drying and storage temperature

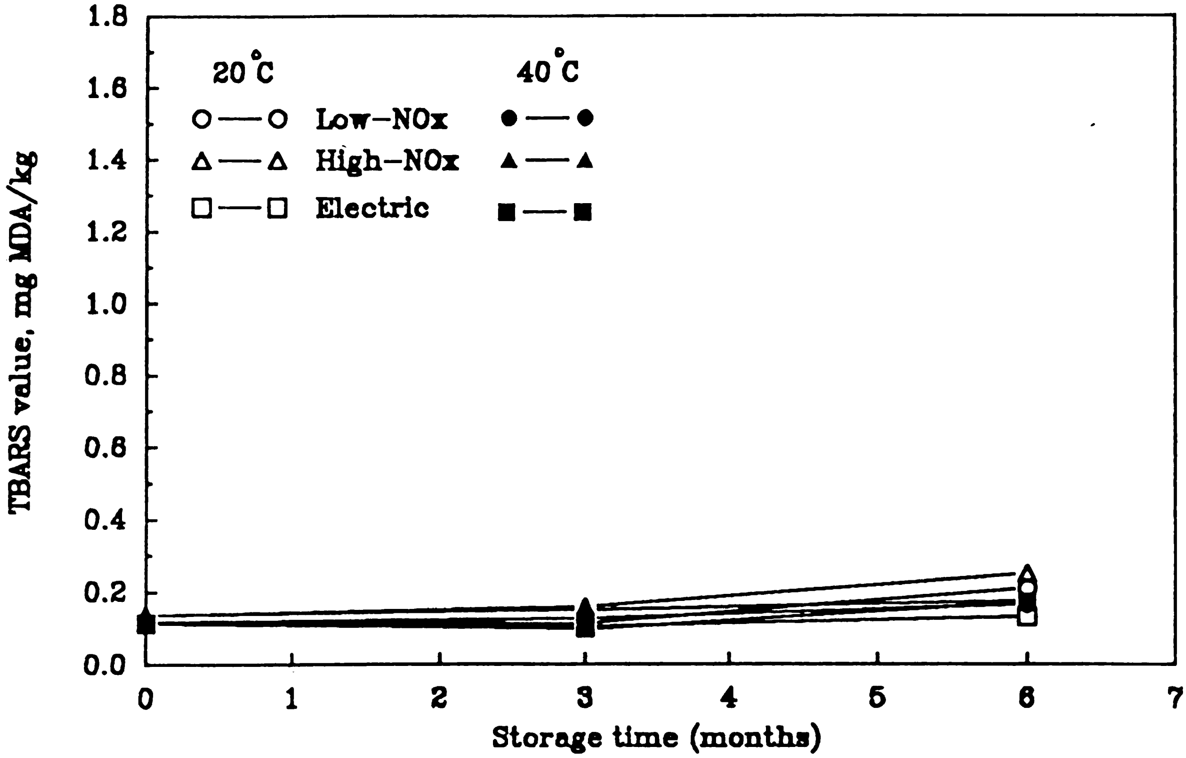


Figure 5. Development of TBARS in whole milk powders in glass vials with oxygen absorbers during storage as influenced by method of drying and storage temperature

An average temperature coefficient (Q_{10}) of 1.55 was reported by Tuohy (1987) for TBARS development in whole milk powders packaged in PE pouches over the temperature range 4-45°C. The magnitude of the Q_{10} value for increase in TBARS values illustrates the profound effect of storage temperature on lipid oxidation in whole milk powders. Although the determination of Q_{10} for TBARS development could not be conducted in this study, general rates of increase of TBARS were obtained by comparing the TBARS values of samples stored at 40°C to those at 20°C. An average factor of 1.64 per 10°C was estimated for TBARS development in the samples packaged in PE pouches over a 6 month storage period. However, a mean value of 1.01 per 10°C was found for the samples packaged in glass vials without oxygen absorbers, which could indicate an insufficient oxygen content in the headspace. With samples packaged in glass vials in the presence of oxygen absorbers, an average value of 0.48 per 10°C was observed. Because the oxygen absorbers reduced the oxygen content of the headspace to less than 2 %, this limited amount of oxygen, therefore, slowed down the rate of lipid oxidation in whole milk powders.

Influence of drying method on lipid oxidation in whole milk powders

The initial TBARS values of whole milk powder samples produced by the various drying methods were not significantly different from each other (Appendix 2). However, during the storage of these powders, the drying method had a significant effect ($P < 0.05$) on TBARS development. In addition, the interactions between drying method and temperature of storage, drying method and packaging, drying method and storage time, were also pronounced.

As shown in Figures 3 and 4, whole milk powder produced by direct high NO_x gas-fired heating was found to deteriorate at a faster rate than powders from direct low NO_x gas-fired and indirect electric heating systems. Powders manufactured by direct high NO_x gas-fired heating and stored at 40°C had the highest TBARS values. Alternatively, samples produced by indirect electric heating and stored at 20°C showed the least increase in TBARS value over the storage period. After 6 months at 40°C, samples produced by direct high NO_x gas-fired heating and packaged in PE pouches had a TBARS value of 1.41 compared to values of 1.33 and 0.86 for the direct low NO_x gas-fired and indirect electric heating powders, respectively. These data were significantly different ($P < 0.05$). When the samples were packaged in glass vials without oxygen absorbers, the development of TBARS showed a similar pattern, although the values were somewhat lower. However, there was only a relatively small change in TBARS values for powders packaged in the glass vials with oxygen absorbers. In these cases, the influence of drying methods was not significant ($P < 0.05$), even in those samples stored at 40°C.

Using the TBARS value as an index of lipid oxidation, it is apparent that whole milk powder manufactured by electric heating had the best oxidative stability, followed by powders produced by direct low NO_x and high NO_x gas-fired heating. It is assumed that the levels of nitrogen oxides generated during the drying processes are mainly responsible for the difference in lipid oxidation in the powders (Lightsey, 1982; Missler et al., 1985). As discussed earlier, by using the low NO_x drying process, the levels of NO_x in the drying air were substantially lower than those generated by the high NO_x drying procedure. Moreover, powders prepared by direct high NO_x gas-fired heating contained the highest levels of nitrate and nitrite, followed by the samples produced by direct low NO_x gas-fired and indirect electric heating. These results coincide with the rate of lipid oxidation in these samples, the extent of oxidation increasing with increasing nitrogen oxide content (as nitrite and nitrate) in the powders.

The initiation of oxidation in model systems of lipids and cholesterol by oxides of nitrogen has been demonstrated by Kamel et al. (1971) and Rhoem et al. (1971). Smith (1981) also pointed out that a variety of free radicals, including oxides of nitrogen are involved in the initiation of lipid oxidation. Thus, the difference in the levels of nitrogen oxides formed during processing seems to be an important factor influencing the oxidative stability of the whole milk powders in this study.

Influence of packaging on lipid stability

The effect of packaging on the development of TBARS in whole milk powders produced by the various drying methods was significant ($P < 0.05$). TBARS values were greatest in samples packaged in PE pouches, followed by the samples packaged in glass vials without and with oxygen absorbers. Temperature of storage and drying method also showed significant interaction effects ($P < 0.05$) the TBARS development, as described previously.

When whole milk powder was packaged in PE pouches and stored at 40°C, a large increase in TBARS value was noted, irrespective of drying method used (Figure 3). A similar increase in the TBARS value of whole milk powder packaged in glass vials without oxygen absorbers was also observed, although not to the same extent as for powders packaged in PE pouches (Figure 4). This difference was attributed to a lower oxygen content in the headspace of the glass vials. As discussed earlier, the concentration of oxygen in the headspace of PE pouches was almost equal to that in the atmosphere. Therefore, powders packaged in PE pouches were continuously exposed to oxidative attack by atmospheric oxygen. In glass vials, however, the oxidative reaction could be attributed to the oxygen incorporated in the powder particles and the residual oxygen in the headspace. Furthermore, the larger surface area of the PE pouches could provide more access for the interaction of headspace oxygen

and the powdered milk samples (Nawar, 1985).

On the other hand, TBARS development in samples in the packaging system containing the oxygen absorbers did not show the same trend as in those samples packaged in the other two systems (Figure 5). Powders manufactured by indirect electric heating showed a slight decrease in TBARS value after 3 months of storage, following by an insignificant ($P < 0.05$) increase after 6 months storage. A similar trend was also found in samples processed by the direct low NO_x drying method. Although the TBARS values of samples prepared by direct high NO_x drying consistently increased during the storage period, this increase was not significant ($P < 0.05$). Moreover, with the presence of oxygen absorber, the TBARS values of the powders were significantly lower than those of powders in other packaging systems ($P < 0.05$). These results demonstrated that oxygen absorbers effectively retard or delay lipid oxidation in whole milk powders during storage, regardless of the processing method used.

Change in fatty acid profiles of whole milk powders during storage

Fatty acid methyl esters of total lipid extracted from whole milk powders were analyzed by gas liquid chromatography. Fatty acid profiles of whole milk powders over the storage period are presented in Tables 6-11. The initial fatty acid profiles of whole milk powder samples produced by the different drying methods were very similar and generally agreed with the report of Cullinane et al. (1984), except for the concentration of butyric acid. An average concentration of 2.3 % butyric acid was obtained in this study. According to Boley and Colwell (1987), the butyric acid content of milkfat is usually between 3.3 to 3.9 %, but is subject to natural variation. The lower C_4 value obtained in this study could also be due to loss of the methyl ester during sample preparation. For this reason, analysis of C_4 through its butyl ester is recommended (Christie et al., 1984).

Fatty acid profiles of whole milk powders were slightly altered during storage. It was noted that butyric acid was not detectable in all samples after 3 months storage. This result could be attributed to the volatility of butyric acid and its methyl ester, which led to the loss of this compound during analysis. A slight decrease in the percentage of total unsaturated fatty acids after 6 months storage was also observed in all samples, while may be due to oxidation.

It was also observed that the samples packaged in PE pouches and glass vials without oxygen absorbers and stored at 40°C, exhibited a greater decrease in total unsaturated fatty acids than those stored at 20°C. There was a relatively small change in the unsaturated fatty acid profiles of samples packaged in glass vials with oxygen absorbers, irrespective of the temperature of storage. Furthermore, the drying methods appeared to have no influence on fatty acid changes. For this

Table 6 - Fatty acid profiles of whole milk powders produced by different drying methods, packaged in PE pouches and stored for 6 months at 20°C.

Fatty acids(%)	electric			low-NO_x			high-NO_x		
	0 mo	3 mo	6 mo	0 mo	3 mo	6 mo	0 mo	3 mo	6 mo
$C_{4:0}$	2.31	0.00	0.00	2.36	0.00	0.00	2.27	0.00	0.00
$C_{6:0}$	2.43	2.44	2.20	2.23	2.15	2.19	2.18	2.15	2.25
$C_{8:0}$	1.40	1.45	1.37	1.34	1.36	1.35	1.51	1.28	1.39
$C_{10:0}$	2.93	2.95	2.83	2.62	2.81	2.80	2.89	2.61	2.86
$C_{12:0}$	3.00	2.91	3.16	3.10	3.16	3.12	3.18	2.92	3.19
$C_{14:0}$	9.31	10.37	10.76	10.06	10.74	10.55	9.83	10.06	10.77
$C_{16:0}$	30.80	30.51	31.01	29.44	31.16	30.66	29.89	30.42	31.09
$C_{18:0}$	15.40	15.45	15.38	14.90	15.66	15.51	14.91	15.44	15.35
$C_{18:1}$	29.41	30.95	30.39	30.97	30.00	30.95	30.30	31.91	30.26
$C_{18:2}$	2.29	2.31	2.25	2.35	2.25	2.26	2.39	2.55	2.20
$C_{18:3}$	0.70	0.66	0.65	0.63	0.70	0.61	0.66	0.65	0.64
Sat.	67.58	66.08	66.71	66.05	67.04	66.18	66.66	64.88	66.90
Unsat.	32.42	33.92	33.29	33.95	32.96	33.82	33.34	35.12	33.10

Table 7 - Fatty acid profiles of whole milk powders produced by different drying methods, packaged in PE pouches and stored for 6 months at 40°C.

Fatty acids(%)	electric			low-NO_x			high-NO_x		
	0 mo	3 mo	6 mo	0 mo	3 mo	6 mo	0 mo	3 mo	6 mo
$C_{4:0}$	2.31	0.00	0.00	2.36	0.00	0.00	2.27	0.00	0.00
$C_{6:0}$	2.43	1.98	2.27	2.23	2.19	2.21	2.18	2.34	2.24
$C_{8:0}$	1.40	1.23	1.41	1.34	1.67	1.38	1.51	1.47	1.39
$C_{10:0}$	2.93	2.55	2.89	2.62	2.60	2.83	2.89	2.81	2.86
$C_{12:0}$	3.00	2.91	3.23	3.10	2.94	3.17	3.18	3.09	3.20
$C_{14:0}$	9.31	10.25	11.00	10.06	10.31	10.74	9.83	10.45	10.88
$C_{16:0}$	30.80	30.69	31.72	29.44	31.00	31.27	29.89	30.61	31.46
$C_{18:0}$	15.40	16.18	15.77	14.90	17.06	15.85	14.91	15.63	15.72
$C_{18:1}$	29.41	30.65	29.04	30.97	29.47	30.28	30.30	30.34	29.98
$C_{18:2}$	2.29	2.89	2.06	2.35	2.22	1.78	2.39	2.67	1.73
$C_{18:3}$	0.70	0.67	0.60	0.63	0.57	0.50	0.66	0.60	0.54
Sat.	67.58	65.79	68.29	66.05	67.77	67.45	66.66	66.40	67.75
Unsat.	32.42	34.21	31.71	33.95	32.23	32.55	33.34	33.60	32.25

Table 8 - Fatty acid profiles of whole milk powders from different drying methods, packaged in glass vials without oxygen absorbers and stored for 6 months at 20°C.

Fatty acids(%)	electric			low-NO_x			high-NO_x		
	0 mo	3 mo	6 mo	0 mo	3 mo	6 mo	0 mo	3 mo	6 mo
$C_{4:0}$	2.31	0.00	0.00	2.36	0.00	0.00	2.27	0.00	0.00
$C_{6:0}$	2.43	2.35	2.21	2.23	2.24	2.22	2.18	2.19	2.21
$C_{8:0}$	1.40	1.38	1.36	1.34	1.38	1.36	1.51	1.38	1.41
$C_{10:0}$	2.93	2.75	2.80	2.62	2.96	2.79	2.89	2.87	2.96
$C_{12:0}$	3.00	3.15	3.13	3.10	3.29	3.13	3.18	3.15	3.35
$C_{14:0}$	9.31	10.28	10.62	10.06	10.62	10.61	9.83	10.10	11.22
$C_{16:0}$	30.80	30.28	30.64	29.44	30.34	30.67	29.89	30.08	30.83
$C_{18:0}$	15.40	15.77	15.30	14.90	15.09	15.44	14.91	15.88	14.77
$C_{18:1}$	29.41	31.01	31.04	30.97	31.11	30.91	30.30	31.28	30.45
$C_{18:2}$	2.29	2.36	2.26	2.35	2.31	2.22	2.39	2.43	2.17
$C_{18:3}$	0.70	0.68	0.64	0.63	0.67	0.64	0.66	0.65	0.61
Sat.	67.58	65.96	66.06	66.05	65.92	66.22	66.66	65.65	66.75
Unsat.	32.42	34.04	33.94	33.95	34.08	33.78	33.34	34.35	33.25

Table 9 - Fatty acid profiles of whole milk powders produced by different drying methods, packaged in glass vials without oxygen absorbers and stored for 6 months at 40°C.

Fatty acids(%)	electric			low-NO_x			high-NO_x		
	0 mo	3 mo	6 mo	0 mo	3 mo	6 mo	0 mo	3 mo	6 mo
$C_{4:0}$	2.31	0.00	0.00	2.36	0.00	0.00	2.27	0.00	0.00
$C_{6:0}$	2.43	2.39	2.28	2.23	2.28	2.24	2.18	2.49	2.33
$C_{8:0}$	1.40	1.46	1.40	1.34	1.33	1.41	1.51	1.50	1.44
$C_{10:0}$	2.93	2.84	2.90	2.62	2.62	2.89	2.89	3.11	2.95
$C_{12:0}$	3.00	3.09	3.22	3.10	3.09	3.21	3.18	3.18	3.30
$C_{14:0}$	9.31	10.34	10.88	10.06	9.86	10.78	9.83	10.70	8.48
$C_{16:0}$	30.80	30.71	31.21	29.44	30.89	31.07	29.89	30.77	32.14
$C_{18:0}$	15.40	15.74	15.48	14.90	15.77	15.60	14.91	15.10	15.98
$C_{18:1}$	29.41	30.40	29.94	30.97	31.22	30.22	30.30	30.16	30.87
$C_{18:2}$	2.29	2.37	2.06	2.35	2.27	1.97	2.39	2.29	1.97
$C_{18:3}$	0.70	0.67	0.61	0.63	0.68	0.60	0.66	0.69	0.54
Sat.	67.58	66.57	67.37	66.05	65.84	67.20	66.65	66.85	66.62
Unsat.	32.42	33.43	32.63	33.95	34.16	32.80	33.35	33.15	33.38

Table 10 - Fatty acid profiles of whole milk powders produced by different drying methods, packaged in glass vials with oxygen absorbers and stored for 6 months at 20°C.

Fatty acids(%)	electric			low-NO_x			high-NO_x		
	0 mo	3 mo	6 mo	0 mo	3 mo	6 mo	0 mo	3 mo	6 mo
$C_{4:0}$	2.31	0.00	0.00	2.36	0.00	0.00	2.27	0.00	0.00
$C_{6:0}$	2.43	2.33	2.21	2.23	2.48	2.24	2.18	2.35	2.24
$C_{8:0}$	1.40	1.48	1.35	1.34	1.50	1.37	1.51	1.61	1.38
$C_{10:0}$	2.93	2.85	2.80	2.62	2.82	2.82	2.89	2.81	2.82
$C_{12:0}$	3.00	3.06	3.14	3.10	3.11	3.13	3.18	3.15	3.15
$C_{14:0}$	9.31	10.54	10.66	10.06	10.16	10.60	9.83	10.71	10.68
$C_{16:0}$	30.80	30.04	30.71	29.44	29.80	30.86	29.89	30.46	30.80
$C_{18:0}$	15.40	15.14	15.28	14.90	15.78	15.56	14.91	15.45	15.33
$C_{18:1}$	29.41	31.61	30.93	30.97	31.32	30.53	30.30	30.47	30.67
$C_{18:2}$	2.29	2.31	2.26	2.35	2.35	2.25	2.39	2.30	2.27
$C_{18:3}$	0.70	0.66	0.66	0.63	0.69	0.65	0.66	0.68	0.66
Sat.	67.58	65.44	66.15	66.05	65.65	66.58	66.66	66.54	66.40
Unsat.	32.42	34.56	33.85	33.95	34.35	33.42	33.34	33.46	33.60

Table 11 - Fatty acid profiles of whole milk powders produced by different drying methods, packaged in glass vials with oxygen absorbers and stored for 6 months at 40°C.

Fatty acids(%)	electric			low-NO$_x$			high-NO$_x$		
	0 mo	3 mo	6 mo	0 mo	3 mo	6 mo	0 mo	3 mo	6 mo
$C_{4:0}$	2.31	0.00	0.00	2.36	0.00	0.00	2.27	0.00	0.00
$C_{6:0}$	2.43	2.48	2.25	2.23	2.77	2.25	2.18	2.16	2.29
$C_{8:0}$	1.40	1.40	1.36	1.34	1.46	1.37	1.51	1.29	1.42
$C_{10:0}$	2.93	2.80	2.82	2.62	2.69	2.82	2.89	2.59	2.93
$C_{12:0}$	3.00	3.05	3.12	3.10	2.86	3.14	3.18	2.93	3.34
$C_{14:0}$	9.31	10.25	10.62	10.06	9.79	10.62	9.83	10.22	10.92
$C_{16:0}$	30.80	29.28	30.59	29.44	29.76	30.90	29.89	30.33	30.31
$C_{18:0}$	15.40	15.73	15.27	14.90	16.60	15.55	14.91	15.59	14.87
$C_{18:1}$	29.41	31.86	31.13	30.97	30.96	30.42	30.30	31.64	30.88
$C_{18:2}$	2.29	2.47	2.22	2.35	2.42	2.27	2.39	2.61	2.42
$C_{18:3}$	0.70	0.68	0.62	0.63	0.68	0.66	0.66	0.65	0.63
Sat.	67.58	64.99	66.03	66.05	65.93	66.65	66.66	65.11	66.08
Unsat.	32.42	35.01	33.97	33.95	34.07	33.35	33.34	34.89	33.92

reason, the TBA procedure is a more sensitive indicator of the extent of lipid oxidation in milk powders than fatty acid profile determination.

Total Cholesterol Content of Whole Milk Powders

Based on a previous study by Engeseth (1990), a very efficient and reproducible method for total cholesterol determination is the modified method of Adams et al. (1986). This method involves direct saponification of the samples, without derivatization, followed by gas liquid chromatographic analysis using packed columns. The total amounts of cholesterol found in whole milk powders ranged from 0.85 to 0.90 mg/g powder (3.39-3.52 mg/g fat). These results are quite similar to that (4 mg/g fat) presented in the Swedish Food Composition Tables (Anonymous, 1986). However, they are higher than the values (2.0 mg/g fat) reported by Nourooz-Zadeh and Appelquvist (1988a).

Cholesterol Oxidation in Whole Milk Powders

The formation of cholesterol oxides during the storage of dried milk products is not addressed in any detail in the scientific literature. Nourooz-Zadeh and Appelqvist (1988a) examined the occurrence of cholesterol oxides in milk powder products during storage as related to processing technology, however, their research focused on the influence of thermal treatment prior to spray drying. In the present study,

samples of whole milk powders manufactured by the three different spray drying methods were analyzed for the presence of cholesterol oxidation products over the 6 month storage period. The influence of the three spray drying procedures as well as various packaging systems on cholesterol oxidation was investigated.

The identification of cholesterol oxides was based on the relative retention times of the trimethylsilyl ether derivatives of sample components compared to those of synthetic cholesterol oxides. Quantification of the cholesterol oxides was based on peak area measurement of the sample peaks relative to that of the internal standard, 6-ketocholesterol. Since cholesterol is a labile compound and improper treatment during the analytical procedure may generate artifacts (Smith, 1981, 1987), all the analyses were done under reduced light and without interruption.

For the analysis of cholesterol oxides in whole milk powder, milkfat was extracted from the samples. The fat contents ranged from 24.9 to 26.5 %, and are similar to those reported previously for commercially prepared whole milk powders (Kurtz, 1974).

The cholesterol oxide concentrations occurring in whole milk powders during storage as function of packaging and processing are shown in Tables 12, 13 and 14. Treatment (processing, packaging, temperature and storage time) effects, as well as significant interactions among treatments were found ($p < 0.01$). The effects of each individual treatment on the formation of cholesterol oxides are discussed as follows.

Effects of storage conditions on cholesterol stability

The development of cholesterol oxidation products in whole milk powders during storage is illustrated in Figures 6, 7 and 8. Cholesterol oxides were not detected in freshly-processed whole milk powder samples, regardless of drying procedure. These results are consistent with those obtained by Nourooz-Zadeh and Appelqvist (1988a).

The increase in the concentrations of cholesterol oxides in milk powders during storage was significant (Figures 6, 7, 8). Total cholesterol oxide contents in whole milk powders stored at 40°C for 3 months and 6 months were 0.61 and 4.45 %, respectively, when expressed as a percentage of the total cholesterol. This difference is due to the fact that time had a significant influence ($P < 0.05$) on the generation of cholesterol oxides in whole milk powders during storage.

Cholesterol oxides were not generally detected in samples until they were stored for at least 3 months. Some cholesterol oxides were detected in samples stored for 3 months at 40°C, 7-ketocholesterol and the epimeric 7-hydroxycholesterols being the major oxidation products, followed by the isomeric 5,6-epoxycholestanols (Tables 12 and 13). The concentrations of these cholesterol oxides increased with further storage. When the samples were stored for six

Table 12 - Cholesterol oxide concentrations in whole milk powders packaged in PE pouches and held at two temperatures (20° and 40°C) for 6 months.

Storage[*] condition	Cholesterol oxides (μg/g lipid)					Total
	7-keto	7α-OH	7β-OH	α-epo	β-epo	
20°C						
3 month						
E[**]	ND	ND	ND	ND	ND	0.0^{a}
L	1.1	ND	ND	ND	ND	1.1^{a}
H	ND	ND	ND	ND	ND	0.0^{a}
6 month						
E	3.6	0.7	4.9	ND	ND	9.1^{a}
L	3.7	ND	4.8	ND	3.1	11.7^{a}
H	17.5	3.3	10.2	0.8	6.4	38.1^{ab}
40°C						
3 month						
E	8.0	14.5	1.2	ND	3.1	26.8^{ab}
L	9.6	3.6	1.4	ND	4.8	19.4^{a}
H	21.4	8.9	14.3	1.4	13.5	59.5^{bc}
6 month						
E	49.2	5.3	13.4	2.7	27.5	73.6^{c}
L	132.0	25.3	30.7	22.9	112.1	322.9^{d}
H	317.0	56.2	45.7	7.1	113.7	539.8^{e}

ND = Not detected, detection limit 0.1 μg.

[*] No COPs were detected in whole milk powders at 0 month (i.e., immediately after processing)

[**] E = electric, L = low-NO_x, H = high-NO_x

[abcde] Mean values in the column with different superscripts are significantly different ($P < 0.05$).

Table 13 - Cholesterol oxide concentrations in whole milk powders packaged in glass vials without oxygen absorbers and held at two temperatures (20° and 40°C) for 6 months.

Storage[*] condition	Cholesterol oxides (μg/g lipid)					Total
	7-keto	7α-OH	7β-OH	α-epo	β-epo	
20°C						
3 month						
E[**]	ND	ND	ND	ND	ND	0.0^{a}
L	ND	ND	ND	ND	ND	0.0^{a}
H	ND	ND	ND	ND	ND	0.0^{a}
6 month						
E	2.2	0.6	5.5	ND	2.9	11.2^{a}
L	7.6	1.0	6.2	ND	3.4	18.1^{a}
H	12.0	4.8	11.7	ND	4.6	33.0^{ab}
40°C						
3 month						
E	5.4	2.6	3.6	ND	6.8	18.5^{a}
L	15.1	3.2	5.8	2.6	3.8	30.4^{ab}
H	13.6	6.7	9.4	ND	ND	29.7^{ab}
6 month						
E	35.0	8.2	16.6	1.4	21.8	82.9^{c}
L	54.9	14.8	25.5	4.2	39.5	138.9^{d}
H	82.1	22.7	38.7	4.6	55.6	203.5^{e}

ND = Not detected, detection limit 0.1 μg.

[*] No COPs were detected in whole milk powders at 0 month (i.e., immediately after processing)

[**] E = electric, L = low-NO_x, H = high-NO_x

^{abcde}Mean values in the column with different superscripts are significantly different ($P < 0.05$).

Table 14 - Cholesterol oxide concentrations in whole milk powders packaged in glass vials with oxygen absorbers and held at two temperatures (20° and 40°C) for 6 months.

Storage* condition	Cholesterol oxides (μg/g lipid)					Total
	7-keto	7α-OH	7β-OH	α-epo	β-epo	
20°C						
3 month						
E**	ND	ND	ND	ND	ND	0.0[a]
L	ND	ND	ND	ND	ND	0.0[a]
H	ND	ND	ND	ND	ND	0.0[a]
6 month						
E	ND	ND	2.9	ND	ND	2.9[a]
L	ND	ND	4.2	ND	ND	4.2[a]
H	9.4	ND	5.4	ND	ND	14.8[a]
40°C						
3 month						
E	ND	ND	ND	ND	ND	0.0[a]
L	ND	ND	ND	ND	ND	0.0[a]
H	ND	ND	ND	ND	ND	0.0[a]
6 month						
E	1.2	ND	ND	ND	ND	1.2[a]
L	ND	ND	ND	ND	ND	0.0[a]
H	2.5	ND	ND	ND	ND	2.5[a]

ND = Not detected, detection limit 0.1 μg.

*No COPs were detected in whole milk powders at 0 month (i.e., immediately after processing)

**E = electric, L = low-NO_x, H = high-NO_x

[a] Mean values in the column with different superscripts are significantly different ($P < 0.05$).

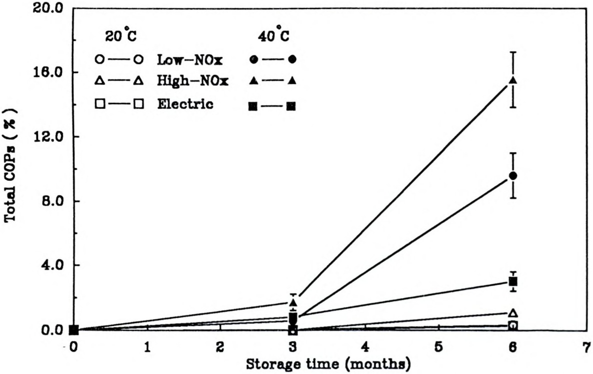


Figure 6. Total cholesterol oxidation products (COPs) (expressed as a percentage of total cholesterol) in whole milk powders packaged in PE pouches as influenced by storage time and temperature

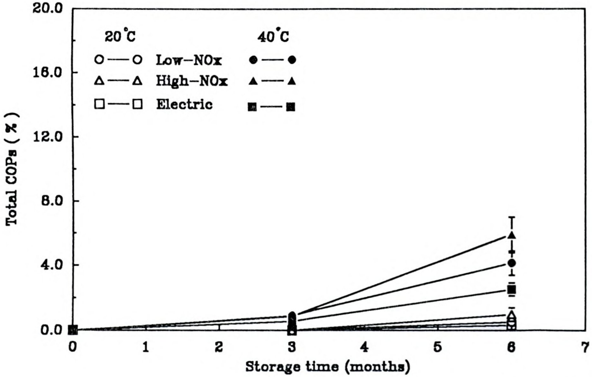


Figure 7. Total cholesterol oxidation products (COPs) (expressed as a percentage of total cholesterol) in whole milk powders packaged in glass vials without oxygen absorbers as influenced by storage time and temperature

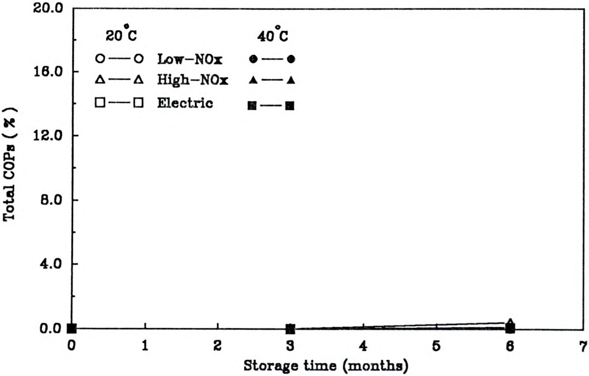


Figure 8. Total cholesterol oxidation products (COPs) (expressed as a percentage of total cholesterol) in whole milk powders packaged in glass vials with oxygen absorbers as influenced by storage time and temperature

months at 20°C and 40°C, all showed an increase in the concentration of individual cholesterol oxides. The predominant cholesterol oxide in all samples was 7-ketocholesterol, which agreed with the observation of Nourooz-Zadeh and Appelqvist (1988a). These researchers reported higher concentrations of 7-ketocholesterol compared to other cholesterol oxides in 12-month-old whole milk powder stored at 20°C. The concentrations of 7β-hydroxycholesterol and β-epoxide were higher than those of 7α-hydroxycholesterol and α-epoxide, respectively. Side-chain hydroxylated cholesterol oxides and 5α-cholestane,3β,5,6β-triol were not detected in the samples after 6 months storage. A similar finding was reported by Nourooz-Zadeh and Appelqvist (1988a).

The data indicate that the ratio of each cholesterol oxide to cholesterol increased during storage. However, on average, the secondary cholesterol oxides did not increase to the same extent as the primary COPs, i.e., those generated through oxidation of cholesterol at C_7. The total concentration of C_7 oxidation products represented 0.63 % and 2.57 % of the total cholesterol in whole milk powder samples stored for 3 and 6 months, respectively. On the other hand, the concentration of secondary cholesterol oxides accounted for only 0.15 and 1.05 % of the original cholesterol in samples stored for 3 months and 6 months, respectively. These results suggest that cholesterol oxidation products at the allylic position, i.e., C_7, will be formed more abundantly

than those at the double bond itself, i.e., positions 5 and 6, in cholesterol of whole milk powder. Smith (1981) noted that this is probably because epoxidation is a secondary process which is dependent on the presence of 7-hydroxycholesterols.

It was observed that 7-ketocholesterol was formed more extensively than any other cholesterol oxide throughout the storage period, especially when the samples were stored at 40°C. The average percentages of 7-ketocholesterol in the total cholesterol oxides were 39.6 and 48.2 % for samples stored at 20°C and 40°C, respectively. According to Smith (1973), thermal decomposition of C_7 hydroperoxides results in the formation of 7α- and 7β-hydroxycholesterol and 7-ketocholesterol. Therefore, it is assumed that at the higher temperature or in the absence of water, the decomposition of the 7-hydroperoxides via dehydration to 7-ketocholesterol is more likely than the formation of epimeric 7-hydroxycholesterols. Moreover, the concentration of 7β-hydroxycholesterol was predominant over the 7α-hydroxycholesterol, as was β-epoxide over the α-epoxide. The ratio of β-epoxide to α-epoxide was approximately 2.9/1, and the ratio of 7β-hydroxy to 7α-hydroxycholesterol was 1.5/1. These data were similar to the findings of Nourooz-Zadeh and Appelqvist (1988a), who reported ratios of 1.6/1 and 1.9/1 for β-epoxide/α-epoxide and 7β-hydroxy/7α-hydroxycholesterol, respectively, in whole milk powders stored for 12 months. These results could be explained by the greater thermodynamic

stability of the equatorial over the axial conformation (Smith, 1981).

Formation of 7-ketocholesterol was nearly linear (r= 0.88) with respect to storage time at 40°C. The formation of 7β-hydroxycholesterol and β-epoxide followed similar trends as well, with correlations of 0.84 and 0.77, respectively. Nevertheless, the linear relationships of storage time and the generation of 7-ketocholesterol, 7β-hydroxycholesterol and β-epoxide did not exist in samples stored at 20°C.

The data presented in Tables 12 and 13 indicate that exposure to a higher temperature during storage caused noticeable concentrations of cholesterol oxides in whole milk powders. The difference between means of temperature treatments was significant ($P < 0.05$). When samples were packaged in PE pouches or in glass vials without oxygen absorbers, the average concentrations of cholesterol oxides in samples stored at 40°C for 6 months were approximately three times greater than those in samples stored at 20°C. Furthermore, the quantities of secondary cholesterol oxides were increased at 40°C. The rate of formation of 7β-hydroxycholesterol was greater than that of β-epoxide at 20°C, however, β-epoxide was formed in higher quantities than 7β-hydroxycholesterol when the samples were stored at 40°C. Because β-epoxide is a secondary oxidation product of cholesterol, it is apparent that the elevated temperature accelerated the oxidative process. Smith (1981) indicated

that the exposure of cholesterol to high temperatures for a relatively long period in the presence of oxygen may initiate the allylic free radical reaction at C_7. Following the formation of C_7 peroxy radicals, a series of free radical reactions take place which results in the formation of stable oxidative products. Thus, storage of samples at 40°C can lead to a more intense oxidative degradation of cholesterol than when stored at 20°C. However, the higher temperature did not have the same impact on the oxidative stability of cholesterol when the samples were packaged in glass vials with oxygen absorbers, which was mainly due to insufficient oxygen in the headspace.

In conclusion, the levels of cholesterol oxides in whole milk powders are greatly affected by temperature and storage time. The rate of oxidation for all cholesterol oxides was greatest between month 3 and month 6.

Effect of drying method on cholesterol oxidation

As mentioned previously, there was no detectable cholesterol oxides in freshly processed whole milk powder samples. These results indicated that the drying method had initially no influence on the presence of cholesterol oxides in fresh products. Similar results were found for samples stored for 3 months at 20°C. Although cholesterol oxides in the samples stored at 40°C were detected after 3 months of storage, the drying methods did not show any significant effect ($P < 0.05$) on the formation of cholesterol oxides.

After 6 months of storage at 20°C, powders prepared by direct high NO_x gas-fired heating had the highest cholesterol oxide content, followed by the samples produced by direct low NO_x gas-fired and indirect electric heating systems. Nevertheless, there was no significant difference ($P < 0.05$) among the means. The methods of drying, however, did significantly ($P < 0.05$) affect the concentrations of cholesterol oxides in whole milk powders stored at 40°C for 6 months.

As shown in Figures 9, 10 and 11, the difference in the total cholesterol oxide contents and the individual cholesterol oxides is related to drying method. Total cholesterol oxides in powders manufactured by the direct high NO_x gas-fired heating process increased rapidly from 59.5 to 539.8 $\mu g/g$ lipid for samples packaged in PE pouches, and from 29.7 to 203.5 $\mu g/g$ lipid for samples packaged in glass vials without oxygen absorbers when stored for 3 months at 40°C. The powders prepared by the direct low NO_x gas-fired heating process followed a similar pattern but contained lower amounts of the cholesterol oxides. There was only a relatively small change in the total cholesterol oxide contents of powders produced by indirect electric heating compared to those from direct gas-fired heating.

The higher concentrations of cholesterol oxides in direct gas-fired whole milk powders can be related to the processing

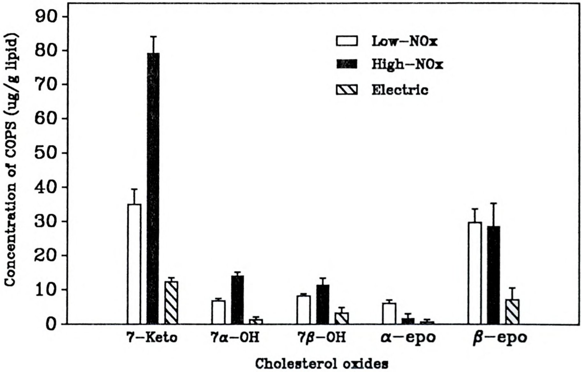


Figure 9. Concentration of cholesterol oxidation products (COPs) in 6 month-old whole milk powders processed by various drying methods, packaged in PE pouches, and stored at 40°C

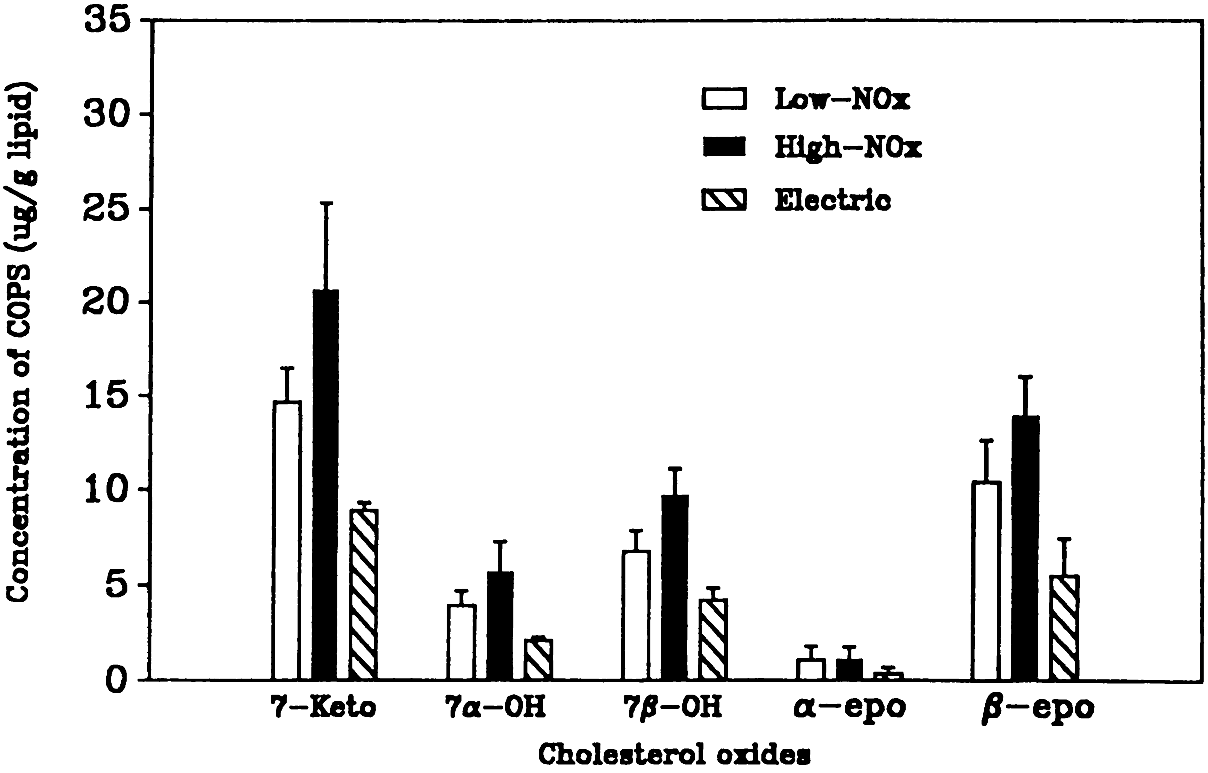


Figure 10. Concentration of cholesterol oxidation products (COPs) in 6 month-old whole milk powders processed by various drying methods, packaged in glass vials without oxygen absorbers, and stored at 40°C

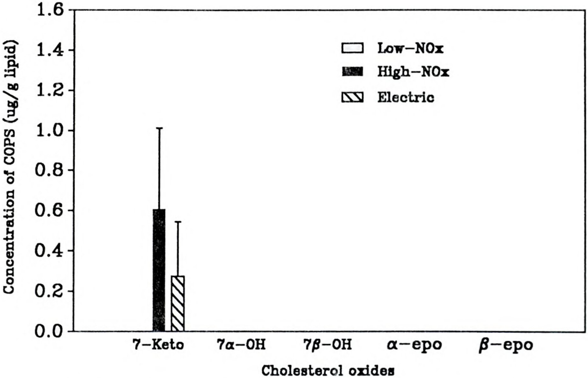


Figure 11. Concentration of cholesterol oxidation products (COPs) in 6 month-old whole milk powders processed by various drying methods, packaged in glass vials with oxygen absorbers, and stored at 40°C

conditions which generated greater quantites of NO_x, known initiators of lipid oxidation. The levels of NO_x in the drying air and the resultant concentrations of nitrite and nitrate in powders prepared by the different drying processes tended to parallel lipid oxidation (as measured by TBARS) and cholesterol oxidation.

Effect of packaging on cholesterol oxidation

The extent of cholesterol oxidation in whole milk powders was related to the packaging conditions, as shown in Figures 12 and 13. The interactions between packaging and processing, temperature and storage time were also significant ($P < 0.01$). It is apparent that samples packaged in PE pouches had the highest concentrations of cholesterol oxides, while those in the glass vials with oxygen absorbers had the least oxides. Total cholesterol oxide concentrations in milk powders processed by direct high NO_x gas-fired heating and packaged in PE pouches increased from 1.7 % of the original cholesterol content after 3 months at 40°C, to 15.6 % after 6 months (Figure 6). In the glass vials without oxygen absorbers, the milk powders processed by the same drying procedure had cholesterol oxide concentrations of 0.86 % to 5.9 % of the original cholesterol after 3 months and 6 months of storage, respectively (Figure 7). In the packaging system with oxygen absorbers, the cholesterol oxidation products accounted for only 0.07 % of the total cholesterol content after 6 months of storage at 40°C (Figure 8).

Considering the interaction of packaging and processing, powders produced by direct gas-fired heating and packaged in PE pouches exhibited the highest concentrations of cholesterol oxides, followed by the samples packaged in glass vials without oxygen absorbers (Figures 12 and 13). Moreover, samples from direct high NO_x gas-fired heating had greater concentrations of cholesterol oxides than those from direct low NO_x gas-fired heating. Samples prepared by indirect electric heating and packaged in PE pouches or in the system without oxygen absorbers achieved moderate levels of oxidation of cholesterol, but there was no significant difference between the mean values at the 95 % confidence level. In the packaging systems with the oxygen absorbers, samples showed the least extent of cholesterol oxidation, irrespective of the drying method used in powder preparation.

Moreover, as discussed above, cholesterol oxides started to appear in detectable concentrations in samples packaged in PE pouches and glass vials without oxygen absorbers after 3 months, and the amounts increased substantially over the next 3 months. On the other hand, the oxygen absorbers effectively prevented oxidative changes in cholesterol during the 6 month storage period. The results demonstrated that the oxygen content in the headspace plays an important role in determining the susceptibility of cholesterol to oxidation, and the formation of cholesterol oxides could be significantly

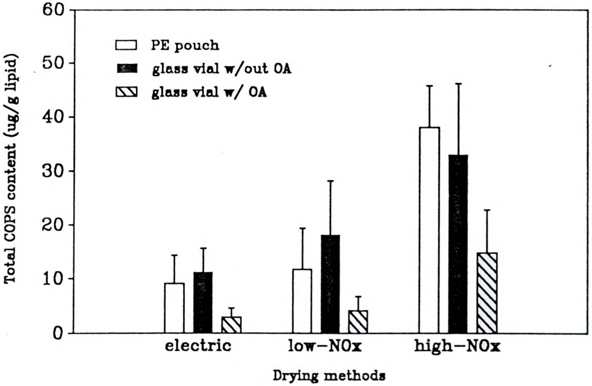


Figure 12. Total cholesterol oxides in whole milk powders processed by different drying methods when packaged in various packaging systems and stored at 20°C for 6 months

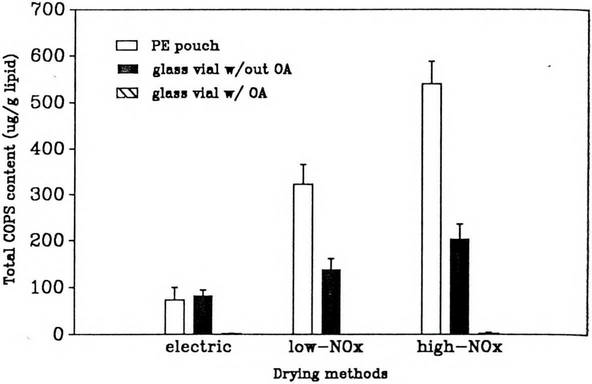


Figure 13. Total cholesterol oxides in whole milk powders processed by different drying methods when packaged in various packaging systems and stored at 40°C for 6 months

minimized by packing whole milk powders with oxygen absorbers.

Correlation of TBARS Values and Cholesterol Oxide Concentrations

The correlation of TBARS values in relation to cholesterol oxide concentrations in whole milk powders is illustrated in Table 15. It is apparent that when the concentrations of cholesterol oxides increased, the TBARS values in the whole milk powders increased linearly for those powders packaged in PE pouches and in glass vials without oxygen absorbers.

Generally, the changes of cholesterol oxide content over storage times agreed with those for the TBARS values for all treatments, except for the samples packaged in glass vials with oxygen absorbers. Correlations of 0.86, 0.82 and 0.79 were found between the TBARS values and the total cholesterol oxide contents in whole milk powders manufactured by direct high NO_x, low NO_x gas-fired heating and indirect electric heating, respectively. These results indicate that the oxidative rate of cholesterol in whole milk powder is proportional to milkfat oxidation under storage and packaging conditions with sufficient headspace oxygen.

Color Change in Whole Milk Powders during Storage

Changes in color (expressed as L, a, b Hunter values) of whole milk powders during storage are listed in Tables 16, 17 and 18. These data indicate that color changes in the samples

Table 15 - The relationship of TBARS values and cholesterol oxide concentrations in whole milk powders manufactured by different drying methods, when packaged in various packaging systems and held at 20°C and 40°C for 6 months.

Storage condition	Packaging system*	Drying method	Best fit regression line**	r
20°C	PE	electric	Y=-0.33+19.31X	0.861
		low-NO_x	Y=-0.32+18.52X	0.755
		high-NO_x	Y=-0.63+34.34X	0.876
	w/out OA	electric	Y=-0.20+13.11X	0.668
		low-NO_x	Y=-0.43+23.76X	0.760
		high-NO_x	Y=-0.70+33.61X	0.835
	w/ OA	electric	Y=-0.14+14.95X	0.700
		low-NO_x	Y=-0.14+12.30X	0.655
		high-NO_x	Y=-0.11+14.15X	0.295
40°C	PE	electric	Y=-0.57+43.86X	0.900
		low-NO_x	Y=-1.67+80.47X	0.920
		high-NO_x	Y=-2.48+112.8X	0.923
	w/out OA	electric	Y=-0.51+44.81X	0.739
		low-NO_x	Y=-0.87+52.20X	0.832
		high-NO_x	Y=-1.23+60.74X	0.789
	w/ OA	electric	Y= 0.02-0.70X	-0.106
		low-NO_x	---	
		high-NO_x	Y=0.035-0.79	-0.051

*PE - polyethylene pouch, w/out OA - glass bottle without oxygen absorber, w/ OA - glass bottle with oxygen absorber.

**X= TBARS value, Y= cholesterol oxide concentration (expressed as a percentage of original cholesterol content).

Table 16 - Changes in color characteristics of whole milk powders prepared by different drying methods, when packaged in PE pouches and stored for 6 months at 20°C and 40°C.

Storage condition	Hunter parameter	Drying methods		
		electric	low-NO_x	high-NO_x
20°C (months)				
0	L-value	92.25	91.38	92.46
3		91.07	90.71	91.02
6		92.36	91.79	92.82
0	a-value	-2.94	-3.18	-3.10
3		-3.00	-3.23	-3.10
6		-3.11	-3.34	-3.30
0	b-value	13.29	15.36	13.63
3		12.91	14.94	13.13
6		13.05	15.10	13.40
40°C (months)				
0	L-value	92.25	91.38	92.46
3		90.88	91.11	91.04
6		91.68	91.17	91.90
0	a-value	-2.94	-3.18	-3.10
3		-3.06	-3.40	-3.48
6		-3.22	-3.49	-3.56
0	b-value	13.29	15.36	13.63
3		12.79	15.15	13.25
6		13.00	14.77	13.44

L: measures lightness and varies from 100 for perfect white to zero for black.

a: measures redness when positive, grey when zero and greeness when negative.

b: measures yellowness when positive, grey when zero, and blueness when negative.

Table 17 - Changes in color characteristics of whole milk powders prepared by different drying methods, when packaged in glass vials without oxygen absorbers and stored for 6 months at 20°C and 40°C.

Storage condition	Hunter parameter	Drying methods		
		electric	low-NO_x	high-NO_x
20°C (months)				
0	L-value	92.25	91.38	92.46
3		90.86	90.59	91.27
6		91.53	90.81	91.65
0	a-value	-2.94	-3.18	-3.10
3		-2.93	-3.27	-3.10
6		-3.11	-3.35	-3.28
0	b-value	13.29	15.36	13.63
3		13.10	15.29	13.33
6		13.24	15.23	13.65
40°C (months)				
0	L-value	92.25	91.38	92.46
3		91.12	90.69	91.01
6		91.61	90.54	91.29
0	a-value	-2.94	-3.18	-3.10
3		-3.08	-3.35	-3.19
6		-3.17	-3.40	-3.28
0	b-value	13.29	15.36	13.63
3		12.97	15.02	13.33
6		13.12	15.05	13.68

L: measures lightness and varies from 100 for perfect white to zero for black.

a: measures redness when positive, grey when zero and greeness when negative.

b: measures yellowness when positive, grey when zero, and blueness when negative.

Table 18 - Changes in color characteristics of whole milk powders prepared by different drying methods, when packaged in glass vials with oxygen absorbers and stored for 6 months at 20°C and 40°C.

Storage condition	Hunter parameter	Drying methods		
		electric	low-NO_x	high-NO_x
20°C (months)				
0	L-value	92.25	91.38	92.46
3		91.36	90.90	91.13
6		91.72	90.91	91.78
0	a-value	-2.94	-3.18	-3.10
3		-2.93	-3.14	-2.93
6		-3.04	-3.26	-3.10
0	b-value	13.29	15.36	13.63
3		13.17	15.17	13.38
6		13.35	15.35	13.59
40°C (months)				
0	L-value	92.25	91.38	92.46
3		91.09	89.87	90.88
6		91.08	89.73	90.72
0	a-value	-2.94	-3.18	-3.10
3		-2.85	-2.92	-2.82
6		-2.97	-2.91	-2.86
0	b-value	13.29	15.36	13.63
3		13.98	16.08	14.53
6		14.33	17.10	15.21

L: measures lightness and varies from 100 for perfect white to zero for black.

a: measures redness when positive, grey when zero and greeness when negative.

b: measures yellowness when positive, grey when zero, and blueness when negative.

were not related to the drying procedures, packaging systems employed, or the temperatures of storage.

The change of color in milk powders is mainly attributed to the nonenzymatic browning reaction (Maillard reaction) between lactose and milk protein (Palombo et al., 1984; Min et al., 1989). High processing temperatures, moisture content and prolonged storage are the major factors involved in the susceptibility of dry milk powders to the Maillard reaction (Bender, 1972; Labuza, 1972). Based on these observations, whole milk powders stored at 40°C were expected to have more brown color than those stored at 20°C. However, the changes in L value (lightness) and b value (yellowness) of the whole milk powders stored at the two temperatures were not significantly different ($P < 0.05$) during the entire storage period. On the other hand, powders produced by the low NO_x drying procedure had significantly ($P < 0.05$) higher b values and lower L values than those produced by the other drying methods, which was unexpected. These results indicated that the Hunter parameters may be not sensitive enough to detect the color changes in the whole milk powders of this experiment. Furthermore, a poor correlation ($r= 0.13$) between b value and TBARS value was observed in all the samples, which suggested that color changes in whole milk powders as measured by Hunter parameters were not related to the extent of lipid oxidation. Min et al. (1989) reported that the brown color in whole milk powder increased with storage time over a 72 hour

storage period at 65°C. Their results demonstrated that measuring the brown color could be a more suitable method for detecting color change in whole milk powders during storage.

SUMMARY AND CONCLUSIONS

The effects of various drying methods and packaging systems on cholesterol oxidation, as well as the relationship between milkfat oxidation and the formation of cholesterol oxides in whole milk powders were the focal points of this study.

Powders manufactured by direct high NO_x gas-fired heating had the highest TBARS values, followed by the powders produced by the direct low NO_x gas-fired and indirect electric heating processes. Lipid oxidation proceeded at a significantly ($P < 0.05$) higher rate in all samples at 40°C than at 20°C, with the powders produced by direct high NO_x drying process undergoing the greatest amount of oxidation.

The method of drying also significantly ($P < 0.05$) affected the concentrations of COPs in whole milk powders. Total COPs in powders produced by the direct high NO_x system increased most rapidly during the storage period, followed by the samples prepared by the low NO_x drying procedure. There was only a relatively small change in total COPs during storage of the powders produced by indirect electric heating. The differences in the TBARS values and the concentrations of cholesterol oxides in powders manufactured by the direct high NO_x, direct low NO_x, and indirect electrically heating processes indicated that nitrogen oxides play an important

role in the initiation of lipid and cholesterol oxidation reactions.

The nature of the packaging systems greatly influenced the oxidative stability of whole milk powders during the storage period. Oxygen absorbers reduced headspace oxygen in the glass vials to approximately 3 % within 3 hours and to less than 2 % after 6 months of storage. Packaging whole milk powders in glass vials with oxygen absorbers was observed to have a protective effect on the stability of milkfat and cholesterol.

TBARS values of powders packaged in PE pouches and in glass vials without oxygen absorbers significantly increased ($P < 0.05$) during the storage period. Samples packaged in glass vials with oxygen absorbers exhibited a much slower increase in TBARS values compared to those packed in the other two packaging systems.

The oxygen absorbers effectively prevented oxidative changes in cholesterol over the 6 months storage period, even in those samples stored at $40^{o}C$. However, cholesterol oxides started to appear in detectable concentrations in powders packaged in PE pouches after 3 months of storage, and the amounts increased substantially over the next 3 months. Similar oxidation products were present in powders packaged in glass vials without oxygen absorbers, but at lower concentrations.

Exposure to the higher temperature facilitated the

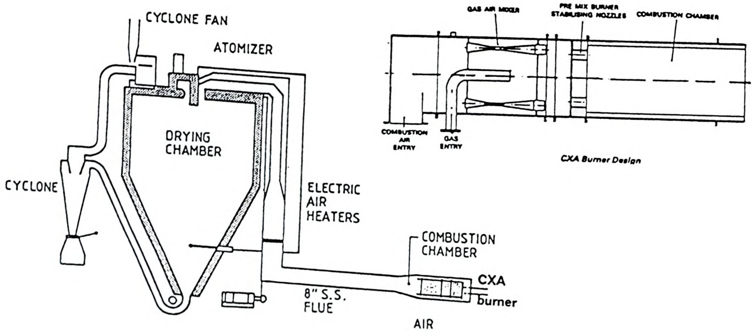
oxidative degradation of cholesterol in whole milk powders. Storage of powders at 40°C led to a more rapid oxidation of cholesterol than in powders stored at 20°C. Formation of 7-ketocholesterol, 7β-hydroxycholesterol and β-epoxide increased linearly with respect to storage time at 40°C.

In general, the rate of oxidation of cholesterol in whole milk powders was parallel to that of lipid oxidation under conditions with sufficient headspace oxygen. A strong linear relationship ($r = 0.82$) was observed between the TBARS values and the total cholesterol oxide contents in whole milk powders packaged in PE pouches and in glass vials without oxygen absorbers over the storage time .

The conclusions drawn from this study are summarized as follows:

1. The large differences in the TBARS values of the powders confirmed that the TBARS value was a satisfactory indicator of lipid oxidation in whole milk powder.
2. The oxidation of lipid and cholesterol in whole milk powders can be minimized by packaging the powders in oxygen-impermeable packages containing oxygen absorbers.
3. Using a direct low NO_x drying process can improve the oxidative stability of whole milk powders.
4. The extent of oxidation of lipid and cholesterol in whole milk powders can be reduced by lowering the storage temperature.

APPENDICES


CXA Burner Design

Appendix 1. Digram of a direct low NO_x CXA gas burner.

Appendix 2 - TBARS development in whole milk powders processed by different drying methods, when packaged in various packagings and stored for 6 months at 20°C and 40°C.*

Storage condition**	electric		low-NO_x		high-NO_x	
	3 mo	6 mo	3 mo	6 mo	3 mo	6 mo
20°C						
PE	0.20±0.01	0.32±0.08	0.23±0.01	0.36±0.06	0.29±0.30	0.45±0.10
w/out OA	0.25±0.01	0.35±0.04	0.26±0.02	0.39±0.06	0.32±0.03	0.47±0.12
w/ OA	0.10±0.02	0.13±0.04	0.11±0.03	0.21±0.03	0.16±0.05	0.25±0.04
40°C						
PE	0.35±0.04	0.86±0.10	0.44±0.04	1.33±0.21	0.65±0.30	1.41±0.36
w/out OA	0.36±0.02	0.56±0.11	0.53±0.17	0.82±0.12	0.62±0.22	0.97±0.08
w/ OA	0.10±0.03	0.17±0.05	0.12±0.02	0.17±0.04	0.15±0.03	0.18±0.05

*The original TBARS in whole milk powders processed by electric, low-NO_x and high-NO_x drying methods are 0.11, 0.12 and 0.13, respectively.

**PE - packaged in polyethylene pouch; w/out OA - packaged in glass vials without oxygen absorbers; w/ OA - packaged in glass vials with oxygen absorbers.

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