This is to certify that the

thesis entitled

THE INFLUENCE OF PACKAGING ON STABILITY
OF DRIED WHOLE MILK

presented by

YUPAWADEE PATTAPANICHCHOTE

has been accepted towards fulfillment
of the requirements for

MASTER degree in PACKAGING

Bruce Haute
Major professor

Date OCTOBER 27, 1993

THE INFLUENCE OF PACKAGING ON STABILITY OF DRIED WHOLE MILK

By

Yupawadee Pattapanichchote

A THESIS

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

MASTER OF SCIENCE

School of Packaging

1993

Dr. Bruce Harte

ABSTRACT

THE INFLUENCE OF PACKAGING ON STABILITY OF DRIED WHOLE MILK

By

Yupawadee Pattapanichchote

Dried whole milk is widely used in the food industry as an ingredient in many food products. Unfortunately, this product undergoes oxidative changes during storage which can result in unacceptable quality. Since oxygen is necessary to cause lipid oxidation, reduced oxygen atmosphere packaging can be used to protect dried whole milk against milk fat oxidation. Oxygen absorbers and vacuum/gas flush packaging were investigated to determine their abilities to prevent oxidative changes in dried whole milk stored under two conditions, 21°C/50%RH and 37°C/90%RH, for 6 months. Package headspace oxygen analysis and lipid and cholesterol oxidation measurements were performed on the stored product. Headspace oxygen within the laminated PVDC pouch, packed with oxygen absorber was reduced to 0.19% at 37°C/90%RH. There were positive correlations between extent of oxidation (TBARS number) and storage time (r=0.95, by average) and between total cholesterol oxides and TBARS number (r=0.93, by average). High temperature storage significantly increased lipid and cholesterol oxidation in dried whole milk. Reduced oxygen atmosphere packaging with oxygen absorber had a protective effect on the lipid and cholesterol components.

ACKNOWLEDGEMENTS

I would like to express my appreciation and thanks to Dr. Bruce R. Harte for his counsel and guidance throughout this study. I wish to thank Dr. J. Ian Gray and Shu-Mei Lai for their valuable instruction and advice in food laboratory. I also would like to thank Dr. Jack Giacin and Dr. John A. Partridge for serving on my committee and MMPA (Michigan Milk Producers Association, Ovid, MI) who provided the dried whole milk samples.

I am indebted to my friends, Dr. Vittratorn Chirapravati and Orla Nithivasin. Without their support, this project could not have accomplished. I am especially grateful to my family for all their love and inspiration.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Page

INTRODUCTION

Dried whole milk has a shelf life of less than 6 months (Kurtz et al., 1970; Boon et al., 1976) because of its susceptibility to oxygen. The oxidation of milk fat has a number of adverse effects on nutritional quality, flavor, and color. In addition, there is growing concern over the presence of cholesterol oxide products (COPs) in food products due to their atherogenicity and inhibition of cholesterol biosynthesis (Peng and Morin, 1991; Addis, 1986). Other biological effects associated with the intake of COPs are carcinogenicity (Bischoff, 1969) and mutagenicity (Sevanian and Peterson, 1986).

As an unsaturated lipid, cholesterol is susceptible to oxidation in the presence of molecular oxygen via a free radical process which can result in the formation of toxic oxidation products (Nourooz-Zadeh and Appelqvist, 1988). Sander et al. (1989) concluded that processing effects, storage conditions, and cholesterol content influence the presence of COPs in food products. Addis and Park (1991) pointed out that longer storage times contribute to higher levels of COPs in stored milk powders.

In contrast, Flanagan et al. (1975) and Finocchiaro and Richardson (1983) reported no quantifiable levels of COPs in milk powder products. Kim and Nawar (1992) reported that in the dry state, nonpolar lipids and the nonlipid fraction

in the milk fat globule membrane (MFGM) protected cholesterol against oxidation. Low or undetectable levels of COPs in milk products may be due to: (1) the modest cholesterol content of milk; (2) the relatively low content of unsaturated fatty acids in milk (Swaisgood, 1985); (3) the sensitivity of the analytical methods for isolation and quantification of COPs in foods such as dairy products (Smith, 1981); and/or (4) gradual reduction of COPs during storage (Addis and Park, 1991).

To minimize COPs formation in milk powders, products can be protected during food processing and by packaging. Use of antioxidants can help prevent milk fat oxidation. Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and propyl gallate (PG) are very effective. However, use of antioxidants may not be the most ethical way to protect products against lipid oxidation because they have to be mixed into the products at the limited concentrations. In general, the total concentrations of permitted antioxidants, added singly or in combination, must not exceed 0.02% by weight based on the fat content of the food (Nawar, 1985). One of the major causes of lipid oxidation is headspace oxygen which reacts with unsaturated fat to form oxidative products. Therefore, completely eliminating the headspace oxygen in the food package may be a more effective way to prevent lipid oxidation.

Vacuum packaging and gas flushing can well remove headspace oxygen from packages. However, even with these techniques, small amounts of oxygen may remain in food pores and channels. Moreover, it is nearly impossible to completely prevent all oxygen from permeating through the packaging material during storage.

Recently, oxygen absorbers (OA) have been commercialized in Japan and the United States. Unlike vacuum packaging and gas flushing, OA function through a chemical reaction that can completely remove molecular oxygen from within the package (Abe, 1991). Oxygen absorbing agents are contained in small pouches or labels made of low barrier material, and are not incorporated directly into the food. The OA pouch is packed along with the food product inside a container of high barrier material. Chemically, OA function by reacting with headspace oxygen and oxygen which is transmitted through the packaging material. Therefore, use of this technology is likely to improve the quality and acceptability of dried milk products during storage.

The major objective of this study was to determine the effect of several packaging systems on the quality and acceptability of dried whole milk and the formation of cholesterol oxides. In addition, the efficiency with which oxygen absorbers and vacuum gas flush packaging removed oxygen when applied to bulk packages of dried whole milk was evaluated. A final objective was to investigate the effect of

several storage conditions on oxidative changes in dried whole milk.

LITERATURE REVIEW

Milk Lipids

Milk lipids have greater variation than other milk components (Sommerfeldt and Baer, 1986). The composition of the lipids is influenced by breed and seasonal changes (Swaisgood, 1985), but is not markedly affected by dietary lipids because at least some milk lipids are derived from metabolism in the cow's rumen (Garton, 1964). Modification of fatty acids can be done by incorporating triacylglycerol emulsions which are protected by a protein membrane cross-linked with formaldehyde (Eskin, 1990). This approach can protect the lipids from microbial metabolism in the cow's rumen, thus, resulting in a modified composition of fatty acids with greater degree of unsaturation.

Milk fat is present as small fat globules surrounded by a milk fat globule membrane (MFGM). The MFGM layer has an important influence on the stability and deteriorative changes associated with processing and storage of milk. The major component of milk fat is triacylglycerols, comprising up to 95 to 96% of the total lipid, followed by small amounts of diacylglycerols, phospholipids, free fatty acids, and traces of cholesterol esters (Table 1).

The fatty acid composition of bovine milk lipid is very complicated. Esterified with glycerols to form

triacylglycerols, the long-chain fatty acids (C14-C23) and short-chain fatty acids (C4-C6) account for 75 and 15% of the total fatty acids in the triacylglycerols, respectively (Eskin, 1990). Table 2 shows the major fatty acids in milk fat. Jensen et al. (1990) reported that bovine milk contains substantial quantities of short-chain saturated fatty acids (C4:0 to C10:0), about 2% of C18:2, and almost no other long-chain polyunsaturated fatty acids (PUFAs). Levels of stearic acid (C18:0) and oleic acid (C18:1) are correlated to each other because some stearic acid is desaturated to oleic acid by rumen microorganisms. Therefore, when either stearic acid or oleic acid is added to dietary feeds, it can increase levels of stearic and oleic acids in milk fat (Christie, 1983).

The PUFAs are mainly associated with phospholipids and are present in the MFGM. However, the phospholipids comprise 1% or less of the total lipid, and contribute small amounts of PUFAs. In addition, dietary PUFAs are biohydrogenated by bovine rumen activity, producing short-chain fatty acids which help keeps milk liquid at room temperature. The fatty acid composition of bovine milk can be modified by feeding a specific diet. Oil rich in linoleic acid (C18:2) and encapsulated with casein can pass through the rumen without being hydrogenated. Milk fat can be modified to contain up to 30% linoleic acid (Hill et al., 1977).

The fatty acid composition of small globules (skim milk fraction) and large globules (cream fraction) is different. There are less C4:0 to C10:0 and C18:0, and more C18:1 in the small globules than in large globules (Timmen and Patton, 1988). Large globules, about 4 μm, make up the largest amount of the total. Large globules of 8 to 12 μm represent only 0.01% of the total fat globules with 1 to 4% of the total lipid. In contrast, the small globules (maximum 1 μm) comprise up to 90% of the total fat globules but contribute only a small weight of the total lipid (Jensen et al., 1990).

Elevated free fatty acids (FFAs) have been attributed to secretion of higher FFA levels and greater lipolysis during processing and storage (Fitz-Gerald et al., 1981). Lipolysis will not change the total fatty acid composition but will alter the relative amounts of FFA, triacylglycerols, diacylglycerols, and monoacylglycerols. Free fatty acids (FFA) tend to associate with the MFGM lipids (Bracco et al., 1972) and become easily oxidized. Therefore, the greater the presence of FFA, the more unstable is the milk fat. Excessive lipolysis causes undesirable off-flavors in milk and milk products. Te Whaiti and Fryer (1976) showed that the levels of FFA varied in normal milks from 0 to 1.3%.

The FFA in freshly drawn milk are very small (Deeth and Fitz-Gerald, 1983; Jensen et al., 1990), but agitation and temperature activation during processing and improper storage can induce lipolysis causing significant increases in

the FFA. However, Anderson (1981) found that peptides in milk (proteose peptones) inhibit lipolysis in bovine milk. According to Lindquist and Brunner (1962), the FFA fraction of spray-dried whole milk contained slightly higher concentrations of neutral glycerides and long-chain saturated fatty acids (C10 to C18) and lower concentrations of C18 unsaturated fatty acids, monoacylglycerols, and phospholipids than the total lipid fraction. FFAs are more susceptible to oxidation than fatty acids esterified with glycerols.

In addition to the major components of milk fat mentioned above, there are significant trace amounts of sterols. Sterols make up about 0.2 to 0.4% of the total lipid and are mainly associated with MFGM. Cholesterol and cholesterol esters are the major sterols. There are other compounds soluble in milk fat such as sphingolipids, fat-soluble vitamins, and flavors.

Table 1 : Milk lipids and relative percentages

Lipid fraction	Percent of total lipid
Triacylglycerols	95-96
Diacylglycerols	1.3-1.6
Monoacylglycerols	0.02-0.04
Keto acid glycerides	0.9-1.3
Hydroxy acid glycerides	0.6-0.8
Free fatty acids	0.1-0.4
Phospholipids	0.8-1.0
Sphingolipids	0.06
Sterols	0.2-0.4

Source: Belitz, H.D. and Grosch, W. (1987)

Table 2: Fatty acid composition of bovine milk fat [a]

Fatty acid	% Weight	
	summer	winter
4:0	3.6	3.5
6:0	1.3	1.4
8:0	0.9	1.1
10:0	2.4	2.7
12:0	2.7	2.9
13:0	0.1	0.1
14:0	9.8	12.7
15:0	1.1	1.0
16:0	25.4	34.4
16:1	0.9	1.3
17:0	0.7	0.7
18:0	15.8	11.6
18:1cis	24.3	19.9
18:1trans	6.4	2.5
18:2+18:3	1.9	1.4

Source: Patton and Jensen (1975)

[a] From Holstein herd

Lipid Oxidation of Milk Fat

Oxidative deterioration of milk fat or other lipid systems is very complicated. Many researchers have studied the effects of free radicals, hydroperoxides, their breakdown oxidation products and their apparent toxicity, carcinogenicity, effect on enzymes, protein, and cellular structure (Frankel, 1984). In addition, secondary oxidation products such as aldehydes, alcohols, acids, and hydrocarbons can decompose to form volatile compounds resulting in off-flavors in milk and milk products. Interaction of various milk components may affect the oxidative mechanism and can have prooxidative and antioxidative effects on milk fat.

There are two pathways which can result in oxidation of milk fat; (1) autoxidation via free radical chain reactions and (2) photosensitized oxidation. For autocatalytic oxidation via free radical chain mechanism, it is unlikely that oxygen reacts directly with the unsaturated fatty acids to form free radicals since the activation energy for such a reaction is rather high (Korycka-Dahl and Richardson, 1980). Therefore, initiation of lipid autoxidation involves potential prooxidants, which catalyze homolytic decomposition of unsaturated fatty acids (LH), resulting in formation of free radicals (L*). These free radicals then react with oxygen to form peroxyl radicals (LOO*). Free radical chain reactions are proceeded by

reacting peroxy radicals with existing unsaturated fatty acids. They then form hydroperoxides (LOOH) and more free radicals which further propagate in the presence of oxygen. Lipid hydroperoxides (LOOH) are the primary products of autoxidation (Frankel, 1991). In summary, free radical autoxidation involves the following 3 steps:

Initiation:	LH	——>	L* + H*
Propagation:	L* + O2	——>	LOO*
	LOO* + LH	——>	LOOH + L*
Termination:	L* + L*	——>	L-L
	LOO* + LOO*	——>	LOOL + O_2
	LO* + L*	——>	LOL
	LOO* + L*	——>	LOOL
	2LO* + 2LOO*	——>	2LOOL + O_2

Figure 1: Mechanism of free radical chain reactions

The rate of lipid autoxidation is dependent on the ease of hydrogen abstraction to form free radicals and is related to degree of unsaturation (Richardson and Korycka-Dahl, 1983), and availability of oxygen. In addition, this autocatalytic oxidation is catalyzed by metal ions, temperature, and light, which function as prooxidants. There is a time interval where no oxidation occurs, called the induction period. The length of induction depends on

temperature, availability of oxygen, prooxidants, and antioxidants (Stansby, 1990).

Copper in the MFGM can catalyze homolytic decomposition of peroxides (Korycka-Dahl and Richardson, 1980) causing propagation of new chains. Heme proteins such as hemoglobin in the MFGM (Bernstein, 1978) and contaminated iron have also been implicated as prooxidants in the onset of PUFA oxidation. Copper has been implicated as being more prooxidant than iron in catalyzing lipid oxidation in milk (Jarrett, 1979).

Metal as initiators:

$$M^{(n+1)+} + LH \longrightarrow M^{n+} + L^{*} + H^{+}$$

$$M^{n+} + O_2 \longrightarrow (M^{(n+1)+}.....O_2^{-})$$

Figure 2: Mechanism of initiation of unsaturated fatty acids catalyzed by metal ions (Chan, 1987)

Metal as prooxidants:

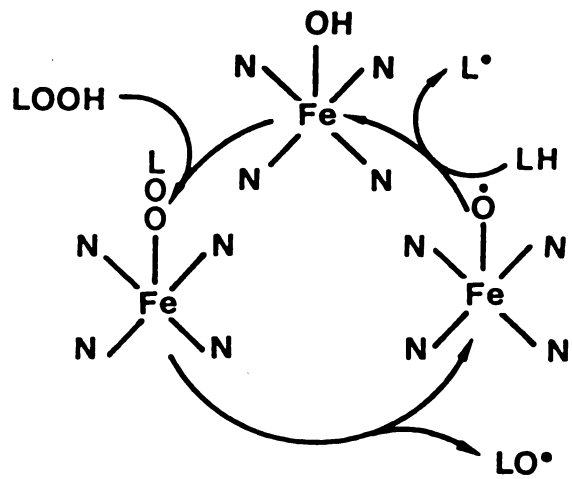
$$M^{(n+1)+} + LOOH \longrightarrow LOO^{*} + H^{+} + M^{n+}$$

$$M^{n+} + LOOH \longrightarrow LO^{*} + OH^{-} + M^{(n+1)+}$$

Figure 3: Mechanism of decomposition of fatty acid hydroperoxides catalyzed by metal ions (Frankel, 1991)

Remark: Metal ions (M^{n+} ,$M^{(n+1)+}$) refer to ions of copper and iron.

Heme-protein as prooxidant:



$LO^{\bullet} + LH \rightarrow LOH + L^{\bullet}$

$L^{\bullet} + O_2 \rightarrow LOO^{\bullet}$

$LOO^{\bullet} + LH \rightarrow LOOH + L^{\bullet}$

Figure 4: Mechanism of oxidation of fatty acid hydroperoxides catalyzed by heme protein (Tappel, 1962)

In addition to the autoxidation mechanism mentioned above, another important pathway of lipid oxidation is photosensitized oxidation (photooxidation). This nonradical mechanism requires a sensitizer (Sens) such as riboflavin, light exposure, and enzymatic catalyst to activate ground state oxygen ($^3O_2$) to singlet oxygen ($^1O_2$) (Figure 5). This excited oxygen is very reactive with the carbon-carbon double bonds of PUFAs. Thus, it is responsible for the potential

initiator of lipid oxidation via a concerted "ene" addition to form allylic hydroperoxides. Subsequently, the resulting hydroperoxides decompose to free radicals contributing to the propagation step in free radical autoxidation. The resulting hydroperoxides from autoxidation and photosensitized oxidation are significantly different in isomeric distributions and concentrations.

$$\text{Sens} \longrightarrow \text{Sens}^* \text{ (excited)}$$

$$\text{Sens}^* + {}^3O_2 \longrightarrow {}^1O_2 + \text{Sens}$$

$${}^1O_2 + \text{LH} \longrightarrow \text{LOOH}$$

Figure 5: Activation of singlet oxygen ($^1O_2$) by a sensitizer (Sens) (Frankel, 1984)

The activated oxygen species includes singlet oxygen ($^1O_2$), superoxide anion (O_2^{*-}), ozone (O_3), hydroxyl radical (HO*), and hydrogen peroxide (H_2O_2). Korycka-Dahl and Richardson (1980) concluded that the most reactive of these activated oxygen species in milk are hydroxyl radicals, singlet oxygen, and ozone. Kurtz et al. (1969) reported that ozone in spray-dried whole milk is detrimental to oxidative stability of milk fat. Xanthine oxidase, which promotes singlet oxygenated superoxide anion formation, is a major prooxidant of lipid oxidation in milk (Hill et al., 1977)

Furthermore, copper in the presence of ascorbic acid forms a copper-ascorbate complex and catalyzes the

formation of activated oxygen species by reducing $^3O_2$ to O_2^{*-}, H_2O_2, or eventually OH*, which can actively oxidize milk fat (Richardson and Korycka-Dahl, 1983)

Natural and synthetic antioxidants such as α-tocopherol, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), function as free radical scavengers, and are able to react with peroxyl radicals (LOO*) to form stable radicals (A*) which are either unreactive or form nonradical products.

$$AH + LOO^* \quad \Longleftrightarrow \quad LOOH + A^*$$

$$A^* + LOO^* \longrightarrow \text{nonradical products}$$

$$A^* + A^* \longrightarrow \text{nonradical products}$$

Figure 6: Antioxidant activity via free radical scavenging (Frankel, 1984)

Photoxidation is not inhibited by free radical scavengers but is inhibited by $^1O_2$ quenchers such as ß-carotene and α-tocopherol. ß-Carotene is the most efficient $^1O_2$ quencher and prevents "ene" addition by $^1O_2$ (Carlsson et al., 1976). Although α-tocopherol exhibits two activities as a free radical scavenger and a $^1O_2$ quencher, it cannot perform both activities effectively because α-tocopherol itself can undergo photooxidation by $^1O_2$ (Clough et al., 1979).

Aurand et al. (1977) studied the effectiveness of scavenging agent (1,3-diphenylisobenzofuran, DPBF) and

quenching agent (1,4-diazabycyclo-[2-2-2] octane, DABCO) in a milk lipid system where lipid oxidation was catalyzed by copper, enzymes, and light. A singlet quencher, DABCO, effectively reacted with singlet oxygen, and as a consequence it inhibited the oxidative reaction involving singlet oxygen. In contrast, the result with DPBF was unexpected because DPBF performed as a very reactive sensitizer rather than a singlet scavenger.

Besides antioxidants, there are other biological species which work against lipid oxidation. Chen and Nawar,(1991) studied the antioxidative effect of saturated phospholipids, dipalmitoylphosphatidylethanolamine (DPE) and dipalmitoyl-phosphatidylcholine (DPC). They found that DPE and DPC exhibited antioxidative effects on milk fat in a dehydrated system. The protective ability of DPE was more effective than that of DPC. The free amino group in DPE may be involved in its antioxidative ability. They also observed the prooxidative effect of MFGM phospholipids.

Cholesterol Oxidation

Cholesterol oxidation in milk products

Cholesterol is a minor component in food and biological systems. Russell and Gray (1979) determined that the cholesterol content of whole milk powders ranged from 2.66 to 3.28 mg/g fat, whereas Nourooz-Zadeh and Appelqvist (1988) reported 2.0 mg/g fat. Chan (1992) reported relatively higher amounts ranging from 3.39 to 3.52 mg/g fat. However, the variation in the total amount of cholesterol in milk powder products may be due to differences in methods of lipid extraction, isolation, and quantification as well as initial composition of milk (Russell and Gray, 1979).

As an unsaturated lipid, cholesterol is susceptible to oxidation in the presence of oxygen, light, and prooxidants via free radical autoxidation to form cholesterol oxidation products (COPs). These COPs have been proven to be highly cytotoxic, atherogenic, and/or carcinogenic (Peng and Morin, 1991). The most atherogenic COPs are 25-hydroxycholesterol and cholestane-triol (Addis, 1986). These two COPs are hardly ever found in fresh milk products.

In general, eight common COPs have been identified in various foods: 25-hydroxycholesterol, 7α-and 7ß-hydroxycholesterol, α- and ß-epoxide, cholestane-triol, 7-ketocholesterol, and cholesta-3,5-dien-7-one which is a thermal degradation product from 7-ketocholesterol (Smith,

1981). On average, food products contain 7-ketocholesterol in the highest amount, followed by α-epoxide, ß-epoxide, and 7ß-hydroxycholesterol (Sander et al., 1989).

Nourooz-Zadeh and Appelqvist (1988) observed that the epimeric 7ß- and 7α-hydroxycholesterols, and 7-ketocholesterol were the major COPs in stored spray-dried skim milk powder, followed by the isomeric 5,6-epoxycholestanols. These COPs were detected at small amounts in the fresh spray-dried whole milk samples. No 20α-hydroxycholesterol or 25-hydroxycholesterol could be observed in fresh milk powder products.

Sander et al. (1988) indicated that fresh dairy products have relatively lower amounts of COPs than processed products. It was proposed that fresh dairy products have lower levels of contaminant transition metals, moderate cholesterol concentration, and relatively high saturated fat levels. Nourooz-Zadeh and Appelqvist (1988) reported that no COPs were detected in freshly-made dairy products at the detection limit of 0.1 ppm (total lipid), for low and medium heat powders. However, detectable levels of COPs were present in the "high heat" fresh spray-dried whole milk and skim milk powders.

In addition, Nourooz-Zadeh and Appelqvist (1988) indicated that spray-dried skim milk powder had a higher cholesterol content compared to other milk products although the total lipid content is about 0.7%, and contained substantial amounts of total COPs (20 to 78 ppm in total

lipid) when stored from 13 months up to 37 months. They reported the total amounts of cholesterol in cream, whole milk, and skim milk powder to be 1.6, 2.0, and 18.9 mg/g fat. This was supported by the work of Russell and Gray (1979). They concluded that for low fat products the cholesterol content of the fat increased as the fat level decreased, while the cholesterol content of the whole product decreased with fat level.

Cholesterol oxidation in other food products

The foods containing the highest levels of COPs were dehydrated egg and meat products (Sander et al., 1989). In contrast, Park and Addis (1987) found that beef, whether freshly cooked or pre-cooked and refrigerated for several days, had very minor amounts of COPs. They also noted that the predominant 7-ketocholesterol found in ground beef and turkey samples, accounted for nearly half of the total COPs, followed by 7ß- and 7∞-hydroxycholesterols.

Dried egg products have been analyzed extensively for COPs because egg yolk contains a high level of cholesterol and iron. Furthermore, dried egg products are exposed to high heat during processing (Tsai and Hudson, 1984). Nitrogen dioxide, generated during combustion, is one of the major causes of cholesterol oxidation (Naber and Biggert, 1985).

Prevention of COPs formation in various foods

Finocchiaro et al. (1984) found high levels of COPs in 4 of 8 brands of grated cheese samples packaged in clear glass containers, but not in fresh ungrated cheese samples. Luby et al. (1986) concluded that butter was highly susceptible to daylight and fluorescent light. Therefore, to prevent oxidative changes in butter, light barrier packaging materials should be employed.

The use of antioxidants is effective in reducing COPs formation. Wade et al. (1986) noted that ascorbyl palmitate (AP), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) were relatively effective in prevention of lipid oxidation in anhydrous milk fat. In addition, elimination of initiators of lipid oxidation such as oxygen and prooxidants such as transition metals and light were highly effective in prevention of lipid oxidation and resulted in improved flavor, color, and safety of the food product. Chan (1992) found that oxygen absorbers effectively prevented oxidative changes in cholesterol during 6 months storage of dried whole milk, at 40°C.

Analytical Methods

TBA test for measurement of lipid oxidation

The 2-thiobarbituric acid (TBA) test is one of the most widely used methods for determining lipid oxidation in lipid-containing foods (Gutteridge and Halliwell, 1990). This chemical test is simple but non-specific. However, it is useful for detecting incipient oxidation of lipids rich in methylene-interrupted, three or more, double bonds (Pomeranz and Meloan, 1987). The TBA test was developed for the evaluation of dairy products (Patton and Kurtz, 1951).

Basically, the test is applied directly to a sample by heating with TBA at low pH, and measured the formation of a chromogen (red pigment) at or close to 532 nm. The chromogen is formed by reaction of two molecules of TBA with one molecule of malonaldehyde (Gutteridge and Halliwell, 1990). Therefore, it was assumed to measure only malonaldehyde, which is a secondary oxidation product of polyunsaturated fatty acids containing three or more double bonds.

However, it was found that other lipid oxidation products such as the 2,4-alkadienals also reacted with TBA to form a red pigment giving the same maximum absorbance as the malonaldehyde-TBA complex at 532 nm (Marcuse and Johansson, 1973). Some compounds such as wood smoke (Dugan, 1955), aldehydes (Patton, 1974), proteins (Gray, 1978), and a

mixture of acetalaldehyde and sucrose (Baumgartner et al., 1975) react with TBA to form color pigments and/or residuals that can interfere with the maximum absorbance. Therefore, the term TBARS (TBA-reactive substances), is a much better term instead of the TBA value or number for indicating the extent of lipid oxidation (Gray and Monahan, 1992).

Furthermore, malonaldehyde detected by the TBA test is not totally formed during the peroxidation process. It is also generated by decomposition of lipid peroxides during the acid-heat treatment. Tarladgis et al. (1964) concluded that acid-heat treatment is not necessary for the condensation reaction of TBA with malonaldehyde, nor for maximum color development.

Generally, the TBA test can be either applied directly to a lipid-containing food without prior extraction of lipid, followed by extraction and measuring of the colored pigments (Sinnhuber et al., 1958) or performed on the steam distillate of the food (Tarladgis et al., 1960). The latter method is more popular because only the free malonaldehyde produced from the oxidative deterioration of the food is measured. Sidwell et al. (1955) mentioned several advantages of the steam distillation method for measurement of oxidation in dried milk products.

Many studies have indicated that the TBA test is not a reliable, accurate, nor specific tool for measuring the lipid oxidation of food products. The method is highly empirical and susceptible to many variables such as pH, time

of heating during distillation and during color development with TBA, stability of pigments, and amount of distillate (Tarladgis et al., 1960) collected. However, the TBA test is simple to apply routinely and provides sensitivity in the measuring of extent of lipid oxidation (Gray and Monahan, 1992). In addition, it has been reported to correlate well with sensory evaluation of oxidized flavors in muscle foods (Igene and Pearson, 1979; Greene and Cumuze, 1981). It is preferable to utilize the TBA test for comparison of samples of a single product at different stages of oxidation, storage, and processing.

Cholesterol oxides determination

Nourooz-Zadeh and Appelqvist (1988) adopted the hexane/isopropanol (HIP) method (Hara and Radin, 1978) for lipid extraction of milk powder products. This method avoids the use of highly toxic solvents, and has reasonably good recoveries. However, Khor and Chan (1985) concluded that the HIP method was not as effective as chloroform/methanol or methylene chloride/methanol methods for soybeans.

Park and Addis (1985a) reported that silica gel column cleanup and HPLC quantification with an internal standard provided nearly 100% recoveries of 7α- and 7ß-hydroxycholesterols, and 7-ketocholesterol from beef samples. In contrast, Higley et al. (1986) reported that using this multi-step cleanup procedure, a poor recovery (23.6%) of total COPs from meat products was obtained. Morgan and

Armstrong (1989) concluded that some COPs may be selectively washed out with the cholesterol fraction during cleanup. They found that cholesterol must be removed prior to analysis because in large amounts (compared with COPs), it can interfere with the quantification of COPs.

Gas chromatography (GC) has been widely used for the resolution of COPs in foods. With the progress in GC technology, highly sensitive capillary columns have been developed and offer superior resolution over packed column GC (Park and Addis, 1991). Using the DB-1 capillary column, epimeric epoxides (Maeker and Unruh, 1986) and 7-ketocholesterol vs 25-hydroxycholesterol (Park and Addis, 1991) can be separated from each other.

The accuracy of COPs analysis is highly dependent on complete extraction of lipid together with a reliable quantification method. It is necessary to develop reliable and sensitive methods of isolation and quantification for COPs in specific food products. The formation of COPs is often present at trace amounts and some food ingredients can interfere with detection. Additionally, extent of cholesterol oxidation is dependent on product composition, contamination, processing conditions, product handling, storage conditions, and storage time.

Oxidized products such as 5-cholest-7-en-3-one (from milk fat) can be artifacts (Smith, 1981). COPs may decompose under some isolation and quantification techniques (Finocchiaro and Richardson, 1983). Nourooz-Zadeh and

Appelqvist (1987) reported that saponification is known to generate several artifacts.

Solid phase extraction (SPE) has been used for COPs cleanup to isolate COPs from triacylglycerols, cholesterol, and other lipids according to their differences in polarity (Park and Addis, 1985b). Packed silica gel columns have been used to isolate COPs from triacylglycerols and other lipids in dried egg products (Tsai and Hudson, 1984). Recently, disposable silica gel columns were used effectively to enrich COPs (Morgan and Armstrong, 1989). Park and Addis (1985a) reported that using silica column cleanup with an internal standard is practical and useful.

Thermal decomposition of some COPs such as 7α- and 7ß-hydroxycholesterols and 25-hydroxycholesterol could occurred during GC analysis (Park and Addis, 1985a). Converting the COPs to their trimethylsilyl ether derivatives prior to GC analysis increases their stability of (Park and Addis, 1985a)

Packaging of Dried Whole Milk

Milk powder should be packaged in materials that are impermeable to oxygen and moisture. Due to unsaturated fatty acids, milk powders will easily oxidize, resulting in the development of hydroperoxides. Hydroperoxides break down to form short-chain aldehydes and ketones, and these compounds are the major causes of the undesirable flavor of dry milk products (Min et al., 1989). Therefore, it is necessary to remove oxygen within the package, prior to storage. In addition, milk powder should be stored in a cool, dark, and dry place to prevent flavor deterioration and decoloration.

The retail packaging of dried milk products is mostly in packages of aluminum foil laminates. Paper-polyethylene-foil-polyethylene laminates may be used for portion packs. For larger retail units, the products may be packaged in metal cans, glass jars, or lined paperboard cartons. Combinations of layers of polyethylene, foil, and paper either for liner or overwrap, with a paperboard carton, will provide a good oxygen and vapor barrier. These types of light-weight packages are designed to supplant glass and metal containers. In addition, packages should have a reclosable closure for the retail package.

Commercial bulk packaging for industrial usage is in barrels, drums, or polyethylene bags inside corrugated

cartons. Under government purchase, it is mandatory to package nonfat dry milk in a polyethylene liner, minimum 3 mm thickness, inside a 3-ply Kraft paper bag with taped-seam over the sewing seal (Hall and Hedrick, 1966). This type of bag (called Type G) is used to prevent insect infestion of the milk products after packaging, usually in 50- or 100-lb bags. After packaging, the bags are stacked on pallets, and stored under low temperature and low humidity conditions.

Gas packaging of dried whole milk should be done immediately after drying to prevent quality deterioration. Vacuum packaging or nitrogen gas flushing under partial vacuum is effective in minimizing the formation of undesirable flavor compounds of dried whole milk during storage (Tamsma et al., 1973).

Findley et al. (1945) observed an improvement in keeping quality of spray-dried whole milk with 0.07% ethyl gallate added to the fluid milk. Therefore, inclusion of vitamin E (as antioxidant) in the diet of bovine animals and/or addition of antioxidants to whole milk before processing will helped reduce lipid oxidation of milk products. Vitamin E (natural antioxidant) is the most effective *in vivo* inhibitor of lipid oxidation (Frankel, 1984).

Antioxidants are free radical scavengers which inhibit the free radical chain reaction. Whereas, chelating agents such as citric acid, and ethyenediaminetetraacetic acid (EDTA) are inhibitors of free radical production in

foods and tie up metal catalysts, copper and iron. These chelating agents are frequently used in combination with antioxidants to provide antioxidant efficiency in food systems (synergistic action).

The total concentration of antioxidants permitted for use in foods must not exceed 0.02% by weight based on fat content (Nawar, 1985). However, use of antioxidants does not seem to be as effective as removal of oxygen. Furthermore, these food additives can cause off-flavors due to their breakdown by-products and may be health hazards (Lindsay, 1985).

The most effective way to protect milk powder against oxidation is to lower the headspace oxygen present in the package. To obtain this purpose, inert gas flushing with partial vacuum is commonly applied. Min et al. (1989) found that dried whole milk samples packaged with 92% N_2 and 8% H_2 in gas-impermeable pouches containing palladium as a catalyst showed significant differences ($P<0.05$) in brown color formation and sensory evaluation from samples packaged with air.

Min et al. (1989) reported that headspace oxygen concentrations of dried whole milk pouches packaged with nitrogen, and mixtures of 92% N_2 and 8% H_2 were approximately 2% (measured immediately after packaging). The residual oxygen could not be easily eliminated by either vacuum or gas flushing, principally due to entrapped air in the milk powder. The amount of oxygen remaining inside the milk

particles after drying was 35% by average (Hall and Hedrick, 1966), and was influenced by content in milk concentrate, spraying pressure and orifice size, treatment of concentrate, and temperature.

Yuki and Wadaka (1972) reported that the oxygen content in a polyvinyl alcohol type laminated film package containing wheat flour chips could be reduced to less than 2% by vacuum and nitrogen gas flushing. Using a packaging material with a high oxygen barrier, a high degree of vacuum in the package can be maintained for a long period of time. In order to obtain and maintain very low residual oxygen concentrations (less than 0.1%) in the package headspace, however, it is necessary to employ an efficient gas flushing system with a scavenging of residual oxygen in the product and to absorb permeating oxygen (Berlin and Pallansch, 1963).

Peters (1974) performed a study using Maraflex® film (American Can Company, Neenah, Wisconsin) for dried whole milk packaging to prevent oxidative off-flavors and color changes. Sample packages were produced with initial oxygen levels of 0.5 to 2.5%. One day after packaging, the residual oxygen was diminished to 0.1-0.2%. Many researchers have evaluated the Maraflex® material for packaging of dried products and/or oxygen-sensitive products. They report that this oxygen scavenger film had protected food products from oxidation, color and flavor changes, and extended product shelf-life. Headspace oxygen concentration was successfully

reduced to less than or equal to 0.1% (Zimmerman et al., 1974; Warmbier and Wolf, 1976).

The multi-layered Maraflex® film (polyester/adhesive/foil/Surlyn®/catalyst/Surlyn®) functions as the oxygen scavenger. The packaging system requires a combination of hydrogen and nitrogen gas (usually 8% H_2, 92% N_2) flushing with a catalyst (palladium or platinum) impregnated in the film structure. The inner ionomer layer, Surlyn® (Du Pont's), has a relatively poor gas barrier property. Therefore, hydrogen and oxygen pass through the Surlyn® layer to the catalyst where they react to form water.

$$2H_2 + O_2 \longrightarrow 2H_2O$$

Figure 7: Reaction developed in the oxygen scavenger system (King, 1955)

The water remains trapped between the two ionomer layers because Surlyn® is a good moisture barrier, whereas the foil and the outer layer (polyester) function as an additional barrier.

Oxygen Absorbers

Oxygen absorbers (OAs) are composed of substrates capable of reacting with oxygen. When placed in a closed container, OAs effectively remove oxygen from within the package. Abe and Kondoh (1989) reported that the headspace oxygen concentration can be lowered below 0.0001% with OA when the proper packaging system is used. As a result, packaged products can be preserved during storage.

OA began to appear commercially in Japan in 1976, and were primarily used with confectionary and bakery products (Kondoh, 1991). Since these products are porous and spongy, it is difficult to remove oxygen headspace to near zero using only physical technologies (vacuum packaging and/or gas flushing). Currently, OAs are being used extensively in the packaging of various kinds of foods, such as coffee (Spaulding, 1988), wine (Carnevale, 1988), beer and beverages (Zenner and Salame, 1989), and microwavable foods (Kondoh, 1991). OAs can maintain an oxygen-free condition within the package for a long period of time (Harima, 1990) and, therefore, can help prevent microbial growth, color and flavor changes, lipid oxidation, formation of toxic oxidized products, and ripening of fruits and vegetables.

By preventing these changes, packaged products will have prolonged shelf life. OAs are not classified as a direct food additive (Abe and Kondoh, 1989). They are economical and

compact in size. The application of OAs in the packaging process is simple, requiring no large mechanical equipment and hence little equipment cost (Harima, 1990). They do not limit productivity due to in-line automatic insertion of OA (Spaulding, 1988) and no waiting time for gas removal or replacement (Abe and Kondoh, 1989). In Japan, a sachet of OA is incorporated into a food package using horizontal/ vertical fold wrap systems, or in preformed bags (Abe, 1991).

Iron powder, ascorbic acid, or catechol is used in most of the existing OAs (Harima, 1990). Iron powder is the main component of most OAs, as ascorbic acid is more expensive. One widely known iron powder-based oxygen absorbers, developed by Mitsubishi Gas Chemical Company, Inc., is under the commercial name of AGELESS®. It is packed in a small sachet in gas permeable materials. Its chemical reactivity with oxygen to form iron rust is as follows:

$$Fe \longrightarrow Fe^{2+} + 2e^-$$

$$1/2\ O_2 + H_2O + 2e^- \longrightarrow 2\ OH^-$$

$$Fe^{2+} + 2\ OH^- \longrightarrow Fe(OH)_2$$

$$Fe(OH)_2 + 1/4\ O_2 + 1/2\ H_2O \longrightarrow Fe(OH)_3$$

Figure 8: Activity of iron powder as oxygen absorber (Abe and Kondoh, 1989).

Oxygen absorber packet under the name of FreshPax® (Multiform Desiccants, Inc., Buffalo, NY) was used to verify

the efficiency of oxygen absorbing within dried whole milk package and, thus, preventing oxidative deterioration of dried whole milk. The packet basically contains ferrous compounds and effectively prevents growth of bacteria, molds, and insect infestation by reducing oxygen residuals as low as 0.05% (Smith et al., 1986). Powers and Berkowitz (1990) had studied an oxygen scavenging packet (OSP) enclosed in a pouch of meal, ready-to-eat bread and reported that OSP effectively prevented growth of a mixed mold inoculum on the bread for 13 months.

There are various types of commercial OAs, developed to meet the specific needs of food manufacturers. Some will absorb oxygen with concomitant generation of carbon dioxide, others absorb not only oxygen but also carbon dioxide simultaneously. In general, OAs can be classified into different categories as presented in Table 3.

Table 3: Classification of oxygen absorbers

Classification	Types of oxygen absorbers
Material	1. Inorganic-iron powder
	2. Organic-ascorbic acid, catechol
Reaction type	1. Self-reaction type
	2. Moisture-dependent type
Reaction speed	1. Immediate effect type
	2. General type
	3. Slow effect type
Usage	1. For very moist food
	2. For moderately moist food
	3. For low-water food
	4. For extra dry food
Function	1. Single function type
	O_2 absorption only
	2. Composite function type
	a. O_2 absorption and CO_2 generation
	b. O_2 absorption and CO_2 absorption
	c. O_2 absorption and alcohol generation
	d. O_2 absorption and others

Source: Harima (1990)

Due to their complex system, OAs must have uniform quality and be able to maintain performance for specific time periods without degradation (Abe and Kondoh, 1989). In addition, they should be enclosed in a barrier package and handled in such a way as to protect OA from absorbing oxygen in the air prior to enclosing within the food package. To maintain such a low oxygen level in the food package, these 3 guidelines should be considered:

(1) Packaging material must have very low oxygen permeability with integrity of closure.

(2) Select the appropriate packet size and type to absorb the oxygen in the headspace within the required time.

(3) Proper packaging and handling systems must be used.

Source: Abe and Kondoh (1989)

Development of new OAs and related technologies include not only consideration of oxygen absorption capacity but also selecting packaging materials with high oxygen barrier, good sealing qualities, and in-line automatic insertion. One unique OA developed by Zenner and Salame (1989) causes oxygen to be tightly bound rather than chemically reacted. This is beneficial since it does not create undesirable by-products or partially reduced oxygen species which could cause off-flavor. This immobilized OA, under the name LONGLIFE® (Aquanautics Corp., Alameda, CA),

retains high affinity for oxygen and has been able to successfully reduce oxygen levels from aqueous solutions down to < 50 ppb. They have developed LONGLIFE® for use in oxygen sensitive beverages such as beer.

There are 4 major concerns when using oxygen absorbers; (1) accidental ingestion, (2) being detected by metal detecters, (3) potential risk from migration of iron oxides into the food (Louis, 1991), and (4) cost (Idol, 1991). To prevent accidental ingestion, affixing OAs firmly to the packaging material or separating OAs completely from the food have been developed. To control cost, Idol (1991) suggested that a lower total system cost may be achieved by working with the supplier. Much research is continuing in this area to solve the remaining problems. Presently, OAs are widely used with various types of food products because of a much wider choice of OAs. Sales are as high as 7 billion packets per year in Japan (Kondoh, 1991).

MATERIALS AND METHODS

Materials

Milk sample

Fresh dried whole milk was obtained from MMPA (Michigan Milk Producers Association, Ovid, MI). The milk powder (28.5% butterfat, labeled by the manufacturer) was prepared by drying in a gas fired spray drying process and packaged in a polyethylene liner inside kraft paper bags, the net weight of each bag was 50 lbs.

A first lot of 10 bags was obtained for the first replicate. One month later, a second lot of 10 bags was obtained for the second replicate. The milk powder was stored at 21°C/50%RH after arrival at the School of Packaging and repackaged immediately into the sample packages.

Packaging materials

Two different materials, low density polyethylene (LDPE) (MidAmerican Plastic) and a laminate film of EVA/PVDC/EVA (ethylene-vinyl acetate/polyvinylidene chloride/ ethylene-vinyl acetate) (Cryovac Co., Duncan, SC) were used in this study. The films were converted into pouches prior to filling with the whole milk powder.

Properties of the LDPE pouch were as followed:

Film thickness: 48.7 μm

Characteristics: gusseted side, pinch bottom

Dimensions (after filling): 20.5cm x 25.5cm x 10.0cm (WxLxD)

Water vapor transmission rate: 1.3-1.5 gm/100in^2/24hrs @ 100°F,90%RH

Oxygen transmission rate: 350 cc/100in^2/24hrs/atm @ 100°F,75%RH

Properties of the laminated PVDC pouch were as followed:

Film thickness: 71.8 μm

Characteristics: 3-side-sealed, flat pouch

Dimensions (after filling): 28.0cm x 30.5cm x 11.0cm (WxLxD)

Water vapor transmission rate: 0.5-0.6 gm/100in^2/24hrs @ 100°F,100%RH

Oxygen transmission rate: 3-6 cc/m^2/24hrs/atm @ 40°F,0%RH

Remark: The information of water vapor and oxygen transmission rates was obtained from the Cryovac Company.

Oxygen absorbers (under FreshPax D2000) were obtained from Multiform Desiccants, Inc. (Buffalo, NY). FreshPax D2000 Oxygen absorber was developed for dry application (Aw < 0.70) to remove package oxygen. Each individual pouch was packaged in a high oxygen permeable material. The absorbers contained an iron based catalyst.

Sample preparation and experimental design

Fresh dried whole milk was vacuum packaged in a laminate (EVA/PVDC/EVA) pouch (2 lb) on the day it was obtained and refrigerated at -20°C for initial analysis (moisture content, lipid oxidation, cholesterol oxide products).

Dried whole milk was repackaged from the 50-lb bags into the sample packages. Five pounds of milk powder were weighed into each packaging system. There were 4 different packaging systems and 2 storage conditions as shown below and in Figure 10.

Packaging systems

1. LDPE pouch (control)
2. Laminated PVDC pouch (control)
3. Laminated PVDC pouch with oxygen absorber (PVDC w/OA)
4. Laminated PVDC pouch with vacuum/gas flushing (PVDC w/V&G)

Storage conditions

1. Room temperature(21±1°C), moderate humidity (50%RH)
2. High temperature (37±1°C), high humidity (90%RH)

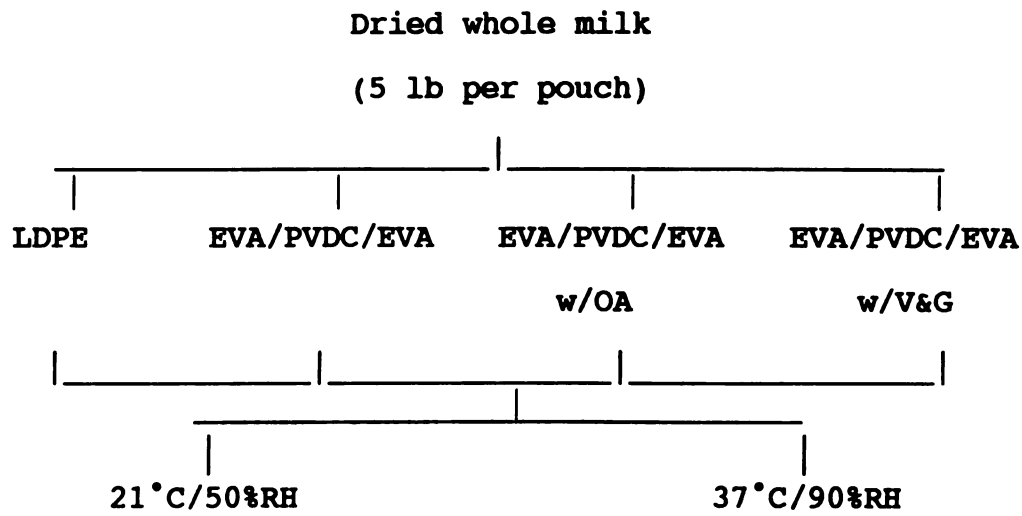


Figure 9: Experimental design used to pack and store dried whole milk

After filling, the sample packages were double heat sealed and placed into corrugated boxes to prevent light exposure. For each replicate, a total of 20 packages of each packaging systems were prepared. Ten packages of each packaging systems were stored at 21°C/50%RH and another ten at 37°C/90%RH, for up to 6 months.

Several analyses and measurements were used to determine the acceptability of stored milk powder samples during 6-months storage:

Analyses and measurements

1. Package headspace oxygen content
2. Moisture content
3. Lipid oxidation (modified TBA test)
4. Cholesterol oxide products (GC analysis)

Package headspace oxygen concentration, product moisture content, and lipid oxidation were performed at the following time intervals: initial time zero, once per month for the first 3 months, and once every 2 weeks for the following 3 months (total 6 months). Cholesterol oxidation products (COPs) were determined initially (zero time), at the third month (3^{rd}), and sixth month (6^{th}).

When sampling, each sample package was shaken and inverted repeatedly before opening. After a package was opened, the milk sample was mixed throughly using a scoop. One scoop of milk powder (about 50 g) was transferred to a dry, clean plastic PE pouch (Whirl-Pak trademark). The pouch was tightly closed and stored in the refrigerator at -20°C for further analysis. During sampling, all analyses and measurements were carried out as quickly as possible (usually less than 2 days) under reduced light, to avoid product deterioration.

In this study, 2 replicates were used, a 1-month time difference between the two was established. For each replicate, a total of 8 sample packages (4 different packaging systems x 2 storage conditions) were analyzed at each designated time interval. From each sample package, duplicate samples were taken and stored in Whirl-Pak PE pouches. Therefore, each analysis and measurement was performed in duplicate for every sample package.

Statistical analysis

The study was conducted as three factors (packaging system, storage condition, and storage time). All analyses and measurements were performed duplicately with two replicates. T-test was used to analyze significant difference between packaging systems because two groups of means were compared at a time.

Methods

Package headspace oxygen analysis

The Headspace Oxygen Analyzer (Model 3500) (Illinois Instruments, Inc., IL) was used to determine package headspace oxygen in the sample packages. Before analysis, the instrument was warmed up for approximately 5 minutes and calibrated to ambient air (~21% O_2) and inert gas (N_2, ~0% O_2).

A septum, a small rubber-like disc with a peel-off backing, was attached directly to the outside of a package. It is used to prevent tearing of the package and to maintain a seal after the needle is inserted. Package headspace (10 ml) was withdrawn from the package through the septa and introduced into the sample loop using a syringe (Illinois Instruments, Inc., IL). The display panel on the headspace oxygen analyzer displays the percent readout of oxygen concentration in the headspace.

Measurement of moisture content

Dried whole milk (3 ± 0.5 g) was weighed into a preweighed aluminum dish on an analytical balance. The dish was placed on a metal rack in a vacuum oven (Model 524) (Precision Scientific, Inc.) for 5 hours at atmospheric pressure. The temperature in the oven was 100 ± 2°C. After 5 hours, the oven was shut off and the dish was removed from the oven to a desiccator using tongs. The dish was cooled to room temperature (about 2 hours), and then reweighed.

The moisture content of the dried milk sample (wet basis) was calculated using the following equation:

$$\% \text{ moisture (wet basis)} = \frac{\text{loss in weight} \times 100}{\text{initial weight of sample}}$$

Measurement of lipid oxidation

Lipid oxidation of the dried whole milk powder was measured using the modified thiobarbituric acid (TBA) method. This modified assay was adapted from Tarladgis et al. (1964), Rhee (1978), and Crackel et al. (1988).

Dried whole milk (10.00 g) was weighed into a 200-ml beaker and dissolved in 95 ml of deionized water. A hydrochloric acid (HCl) solution (2.5 ml) (see Appendix 1), a small quantity of antifoam (Thomas Co., Swedesboro, NJ), and boiling stones (Norton Co.) were added into a dry, clean Kjeldahl flask containing 5 ml of 0.5% PG/EDTA solution (see Appendix 1) as an antioxidant reagent (Rhee, 1978). The milk

powder slurry and its washing solution (5 ml of deionized water) were transferred into the Kjeldahl flask, and swirled to mix the contents.

Using the steam distillation method (Tarladgis et al., 1964), 50 ml of distillate were collected in a capped test tube. Five milliliters of the distillate were then pipetted into a test tube containing 5 ml of aqueous TBA solution (see Appendix 1) (Crackel et al., 1988). For the reagent blank, 5 ml of deionized water were mixed with aqueous TBA reagent instead of the distillate. All tubes were tightly capped, vortexed to mix the contents, and immersed into a boiling water bath for 35 minutes. The tubes were then immediately cooled in a water bath for 10 minutes. A portion of the combined distillate-TBA solution was then transferred into a cuvette to measure its color development. The absorbancy of the pink color was read against the reagent blank at 532 nm (Crackel et al., 1988) using a double beam Bausch and Lomb Spectronic 2000 Spectrophotometer (Rochester, NY).

The absorbancy was corrected by multiplying with the aqueous TBA conversion factor 6.2 (Crackel et al., 1988). The results were reported as TBARS number (thiobarbituric acid-reactive substances number) and used to show the extent of lipid oxidation of dried whole milk during the 6-month storage.

Analysis of cholesterol oxide products

Cholesterol oxide standards

The following cholesterol oxide standards (TLC grade) were purchased from Steraloids Inc. (Wilton, NH): cholesterol (5-cholesten-3ß-ol), 7α-hydroxycholesterol (5-cholesten-3ß,7α-diol), 7ß-hydroxycholesterol (5-cholesten-3ß,7ß-diol),cholesteryl α-epoxide (cholestan-5α,6α-epoxy-3ß-ol), cholesteryl ß-epoxide (cholestan-5ß,6ß-epoxy-3ß-ol), 7-ketocholesterol (5-cholesten-3ß-ol-7-one), 6-ketocholesterol (5α-cholestan-3ß-ol-6-one), 20α-hydroxycholesterol (5-cholesten-3ß,20α-diol), 25-hydroxycholesterol (5-cholesten-3ß,25-diol). The internal standard (6-ketocholesterol) and the standard mixtures were dissolved in ethyl acetate and stored at -20°C, prior to use.

Procedure

Cholesterol oxide products (COPs) in the dried whole milk were quantitatively identified using the modified method of Morgan and Armstrong (1989). Solid phase extraction (SPE) was adopted for COPs cleanup procedure to isolate COPs from triacylglycerols, cholesterol, and other lipids. Because COPs was present at very small amounts, the analysis was done under reduced light and air exposure.

3-ml Supelclean™ LC-Si SPE tubes (Supelco Inc., Bellefonte, PA) were used for extracting COPs from the lipid extract. Supelclean™ disposable solid phase extraction (SPE) tubes contain 300mg silica gel-based, bonded phase packings. Eluting solutions were passed through these SPE tubes by

vacuum using the Extraction Vacuum Manifold (Supelco Inc., Bellefonte, PA).

Since a 3-ml LC-Si SPE tube was not large enough to contain a whole eluting solution at a time, it was joined to a 20-ml reservoir via an adapter. To activate the packing before the sample was extracted, the LC-Si SPE tube was conditioned with 5 ml hexane.

The lipid extract (see Appendix 2) and a 10 μl aliquot of internal standard (6-ketocholesterol, 1.375 μg/μl concentration) were transferred into the LC-Si SPE tube. A 5 ml aliquot of hexane/diethyl ether (95:5, v/v) was used to rinse the empty flask which had previously contained the lipid extract. This was then transferred into the LC-Si SPE tube. Another 5 ml of hexane/diethyl ether (95:5, v/v) was added to elute the triacylglycerols from the silica adsorbent. The remaining triacylglycerols, diacylglycerols, fatty acids, and cholesterol were eluted with 25 ml of hexane/diethyl ether (90:10, v/v) and 15 ml of hexane/diethyl ether (80:20, v/v).

In the final washing step, the cholesterol oxide products were eluted out of the tubes with 10 ml acetone. They were collected in a clean test tube, and the acetone fraction was evaporated to dryness under a nitrogen stream (do not overdry). The oxides were derivatized in 50 μl pyridine (Pierce Chemical Co., Rockford, IL) and 50 μl BSTFA [N,O-Bis(trimethysilyl)trifluoroacetamide] (Pierce Chemical Co., Rockford, IL), in a 1-ml vial closed with a screw cap.

The reaction proceeded at room temperature (in the dark) for 30 minutes to form trimethylsilyl ether derivatives of the COPs. The mixture was dried under nitrogen and redissolved in 100 μl hexane for GC analysis.

A Hewlett Packard Gas Chromatograph Model 5890A (Avondale, PA) equipped with a flame ionization detector (FID) was used for analysis of COPs. COPs were separated and identified using a fused silica capillary column DB-1, 15 m x 0.25 mm i.d. column, 0.1 μm film thickness (J & W Scientific, Inc., Ann Arbor, MI). The column oven temperature was programmed from 170°C (initial temp) to 220°C (final temp) at 10°C/min, then increased to 236°C (temp A) at 0.4°C/min (rate A), and 320°C (temp B) at 10°C/min (rate B) which was then held for 25 min (time B). The injection temperature was 275°C; the detection temperature was 300°C. Carrier gas (Helium) at 50 psi head pressure was delivered at a flow rate of 27 ml/min.

Identification of COPs was based on the relative retention times of trimethylsilyl ether derivatives compared to those of cholesterol oxide standards assayed under identical conditions. A response factor (RF) of each cholesterol oxide component was calculated from its peak area relative to the peak area of the internal standard (6-ketocholesterol). Quantification of COPs was calculated by multiplying their response factors with their peak areas. The amount of each cholesterol oxide (μg) was reported based on 1 gram of milk lipid (see Appendix 3).

RESULTS AND DISCUSSION

Headspace oxygen concentrations in different packaging systems

To examine the efficacy of the oxygen absorber to eliminate headspace oxygen within the package, headspace oxygen concentrations in the packaged samples were measured during a 6-month storage period. The initial oxygen concentrations within LDPE pouches, laminated PVDC pouches, and laminated PVDC pouches with oxygen absorbers (PVDC w/OA) were 20.6-20.8%, whereas laminated PVDC pouches subjected to vacuum and nitrogen gas flushing (PVDC w/V&G) were 1.95-2.25%. All measurements were taken immediately after filling and sealing of the packages. These numbers are typical for vacuum/ gas flushing techniques. Vacuum and nitrogen gas flushing can lower the internal oxygen concentration to below 0.5% (Abe and Kondoh, 1989). However, because of the porous character of milk powder, it was difficult to reduce headspace oxygen concentrations to this level.

In the PVDC w/OA packaging system, the oxygen concentrations were reduced to 1.53-2.10% (21°C/50%RH) and 0.95-1.84% (37°C/90%RH), one day after packing. In the PVDC w/OA system, the oxygen absorbing ability of the OAs was more efficient at 37°C/90%RH than at 21°C/50%RH (Figure 10 and 11). The higher temperature possibly accelerates OA reactivity. Harima (1990) indicated that change of oxygen-

absorbing speed of iron type oxygen absorber depends on temperature.

The lowest oxygen concentration recorded was in a PVDC w/OA pouch (0.19%, at 37°C/90%RH), after one month storage (Figure 11). Whereas, the lowest oxygen concentration in the same packaging system (PVDC w/OA) at 21°C/50%RH was 1.02%, after one month storage (Figure 10). The porous character of milk powder and bulk packaging of the samples (5 lb per package) are factors that could reduce the OA efficiency. Oxygen concentrations within PVDC w/OA packaging systems remained at low levels, 2.42% at 21°C/50%RH and 2.21% at 37°C/90%RH, after 6-month storage. Gradual increases in oxygen concentrations were probably due to oxygen permeating through the packaging material and/or the seal.

The headspace oxygen concentrations in PVDC pouches (without OA) gradually decreased during storage, whereas oxygen concentrations in LDPE pouches were rather constant at the same levels (Figure 10 and 11). The poor oxygen barrier quality of the LDPE material allows oxygen to permeate through the material during storage. For PVDC barrier pouches (without OA), the decrease in oxygen concentration within the package at 37°C/90%RH is assumed to be due to the reaction of oxygen with milk fat particularly when exposed to high temperature.

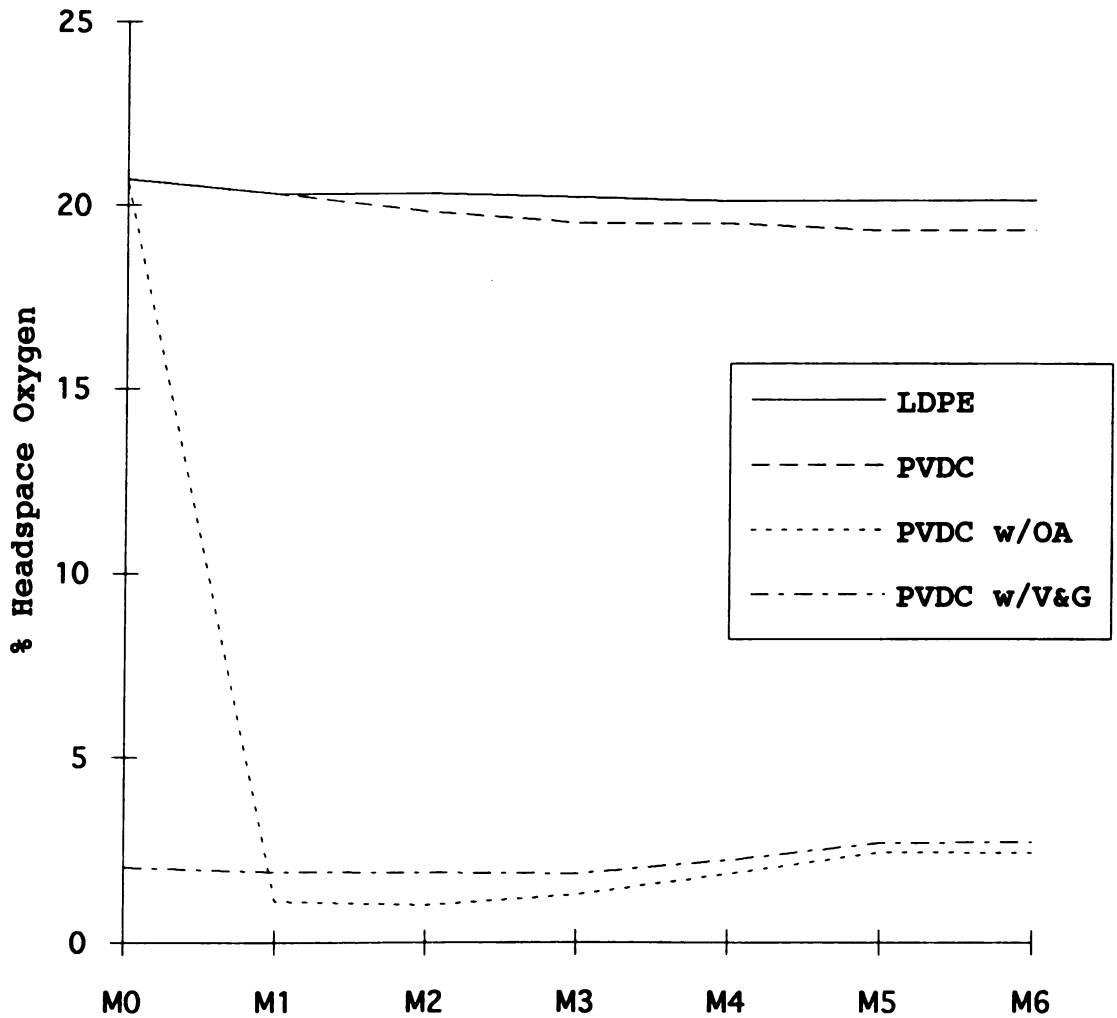


Figure 10: Headspace oxygen concentrations of dried whole milk stored at 21° C/50%RH for 6 months

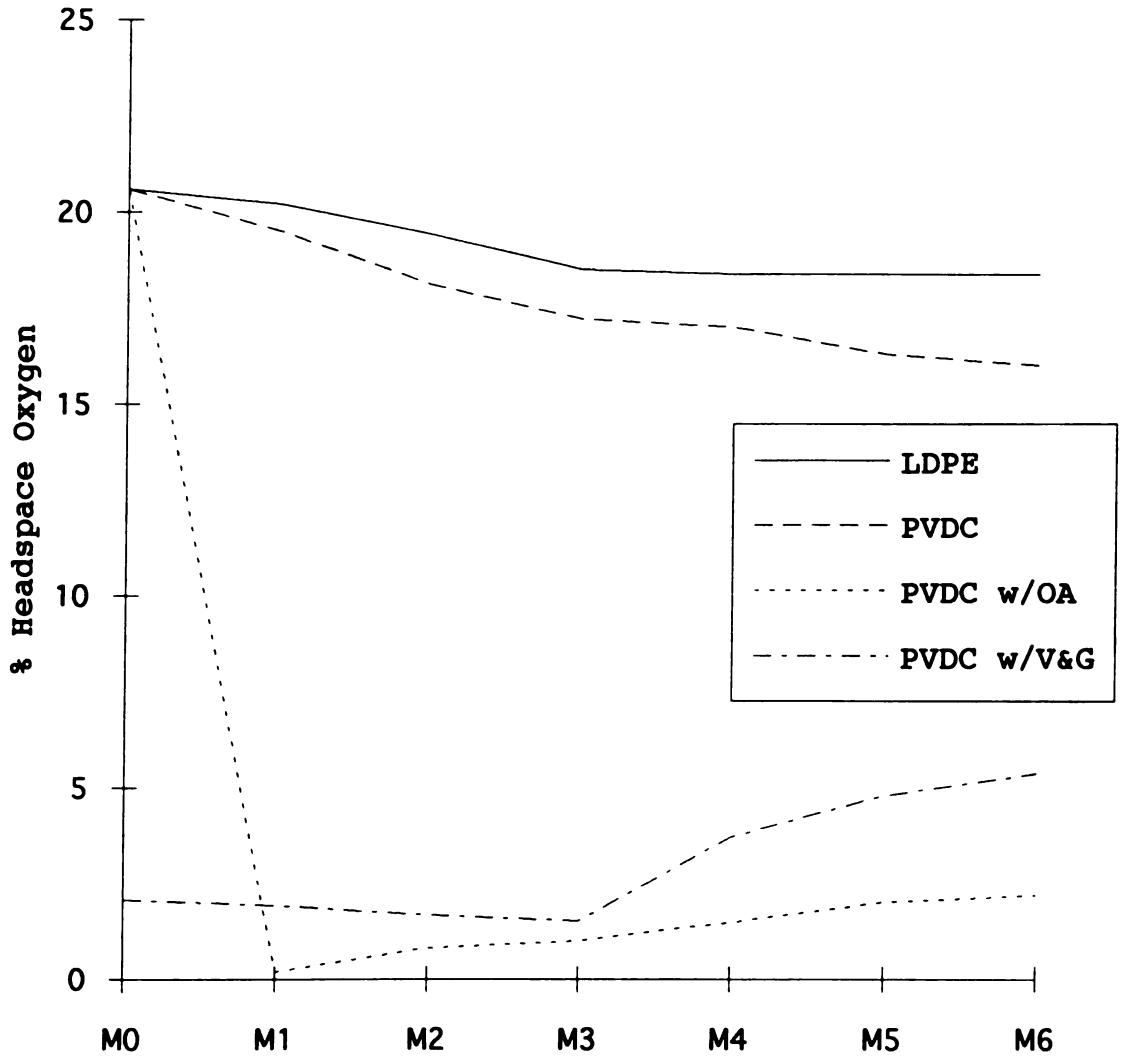


Figure 11: Headspace oxygen concentrations of dried whole milk stored at 37° C/90%RH for 6 months

Moisture content of dried milk samples packaged in different packaging systems

The initial moisture content of the dried whole milk was 3.49%. This number is below the moisture content standards (3.5%) for dried whole milk (Belitz and Grosch, 1987). Moisture contents can vary due to the use of different analytical methods. Moreover, within one analytical method, moisture content can fluctuate depending on atmospheric change, oven temperature, air circulation, and location of sample.

Samples packaged in LDPE pouches had the highest moisture contents, 4.19% (at 21°C/50%RH) and 6.81% (at 37°C/90%RH), after 6-month storage (Table 4). There was a significant difference (P=0.05) in moisture content of samples packaged in LDPE and PVDC pouches when stored at both conditions for 6 months. Therefore, the packaging materials (LDPE and laminated PVDC films) used did affect moisture content of dried whole milk.

There were no significant differences (P=0.01) in moisture content of those samples packaged in PVDC, PVDC w/OA, and PVDC w/V&G, when stored at either storage condition for 6 months. Therefore, gas flushing or packaging with OAs does not effect moisture content of dried whole milk samples.

Storage in high relative humidity caused significant increase in moisture content in dried whole milk samples even for those packaged in the PVDC pouches. Several deteriorative reactions are influenced by product moisture

Table 4 : Moisture content (%) of dried whole milk samples at 2 storage conditions for 6 months

Storage Cond.	Pkg. System	Moisture Content (%)						
		Stroage time (months)						
		M0	M1	M2	M3	M4	M5	M6
21 C/50%RH	LDPE	3.49	3.66	4.03	4.13	4.17	4.19	4.19
	PVDC	3.49	3.53	3.53	3.54	3.55	3.52	3.54
	PVDC w/OA	3.49	3.51	3.50	3.54	3.53	3.54	3.53
	PVDC w/V&G	3.49	3.54	3.52	3.54	3.51	3.51	3.53
37 C/90%RH	LDPE	3.49	4.51	6.04	6.45	6.78	6.81	6.81
	PVDC	3.49	4.02	5.44	5.47	5.57	5.57	5.56
	PVDC w/OA	3.49	4.03	5.50	5.57	5.64	5.63	5.62
	PVDC w/V&G	3.49	4.02	5.46	5.57	5.61	5.61	5.60

content. Water absorption can greatly influence quality changes of dried milk products, especially staleness and browning (Hall and Hedrick, 1966).

For the measurement of lipid oxidation, the results were reported as TBARS number. The relationship between moisture content and TBARS number (the extent of lipid oxidation) of dried whole milk packed in different packaging systems was investigated. Relatively strong correlations were found in the LDPE pouched products ($r = 0.809$, at 21°C/50%RH and $r = 0.903$, at 37°C/90%RH). For the PVDC (without OA), PVDC w/OA, and PVDC w/V&G packaging systems, correlation coefficients between moisture content and lipid oxidation ranged from 0.077 to 0.781. Correlation coefficients varied over a wide range because of the difference in oxygen levels in the package headspace. This means that increased moisture content in the presence of unlimited oxygen resulted in increased oxidation.

Oxidative changes in dried whole milk samples packaged in different packaging systems

The extent of lipid oxidation of dried whole milk samples during 6-months storage was measured using the modified TBA test and reported as "TBARS number" (thiobarbituric acid-reactive substances number) (Crackel et al., 1988). Many researchers have questioned the nonspecific nature of the TBA test. In this study, the test was used to determine the extent of lipid oxidation of a single product

packaged in different packaging systems, and was performed in a consistent manner. Therefore, the data generated should provide a useful means by which to evaluate the protective effects of various packaging systems. Tuohy (1987) concluded that the TBARS number was an effective index of milk fat oxidation, compared to other measurements such as peroxide value, oxygen absorption level, or sensory scores.

The initial TBARS number of dried whole milk samples was 0.010. After 6-month storage, samples packaged in LDPE pouches and stored at 37°C/90%RH had the highest TBARS number of 1.252 (Figure 13). This was due to the poor oxygen barrier quality of LDPE (unlimited oxygen) and the severe storage condition that accelerated the oxidative reaction.

In contrast, the lowest TBARS number, 0.129 after 6 months, was in the PVDC w/OA system, stored at 21°C/50%RH (Figure 12). The TBARS number in the PVDC w/V&G system, stored at 21°C/50%RH was 0.194 (Figure 12). The package headspace oxygen of PVDC w/OA was 2.42% and that of PVDC w/V&G was 2.70%, after storage for 6 months at 21°C/50%RH. The difference in lipid oxidation between these 2 packaging systems when stored at both conditions was significant at P=0.05. This indicates that the oxygen absorbers provided more protection and helped maintain lipid stability, compared to vacuum and gas flush packaging.

Data in Table 5 show the relationship between TBARS numbers and storage time of dried whole milk samples and their correlation coefficients (r), TBARS numbers increased

in a positive linear manner with storage time. Correlation coefficients, ranged from 0.881 to 0.995, indicating that TBARS number was strongly correlated with storage time. The lowest correlation coefficient (0.881) was found in the PVDC w/OA system, stored at 37°C/90%RH, which is higher than results reported by Chan (1992). She reported a correlation coefficient of 0.55 in samples packaged in glass vials with OA and stored at 40°C.

Table 5: Correlation coefficients for TBARS numbers and storage time of dried whole milk samples packaged in different packaging systems for 6 months.

Storage Cond.	Pkg. System	Best fit regression line[a]	Correlation coefficient(r)
21°C/50%RH	LDPE	Y = -2.46e-2 + 7.53e-2X	0.986
	PVDC	Y = -4.21e-3 + 5.29e-2X	0.995
	PVDC w/OA	Y = -6.86e-3 + 1.90e-2X	0.940
	PVDC w/V&G	Y = -2.03e-2 + 2.93e-2X	0.920
37°C/90%RH	LDPE	Y = 4.32e-2 + 0.20X	0.995
	PVDC	Y = -3.21e-2 + 0.12X	0.994
	PVDC w/OA	Y = -1.05e-2 + 4.19e-2X	0.881
	PVDC w/V&G	Y = -5.54e-2 + 7.96e-2X	0.911

[a]When X = storage time (months) and Y = TBARS number

TBARS numbers of product packaged in PVDC w/OA at 37°C/90%RH increased during the last two months (Figure 13). After 4 months, the OAs will have less oxygen absorbing

ability. The headspace oxygen concentrations in the packages increased during this period of time also (Figure 11). Thereafter, oxygen that permeated through the packaging material would rapidly react with milk lipids. The higher temperature can cause increases in oxygen and water vapor transmission rates. In addition, exposure to high temperature for a long time could possibly degrade the barrier properties of the packaging material causing increased levels of oxygen and moisture.

OAs provided substantial protection of the lipids in dried milk samples for up to 4 months, even at 37°C/90%RH. OAs will provide maximum protection when: (1) they are incorporated within high oxygen barrier packages; (2) packages with seal integrity; (3) appropriate type and size OAs are used; and (4) in products stored at moderate condition and appropriate storage time. The above is supported by the results for product stored in the PVDC w/OA system at 21°C/50%RH (Figure 12).

Similar results were observed in TBARS numbers for the PVDC w/V&G system at 37°C/90%RH. There was a sharp increase in TBARS numbers during the last 3 months (Figure 13). In this technique, the packaging material (laminated PVDC film) was treated under high negative pressure, possibly causing degradation of the barrier properties of the film. The increasing level of headspace oxygen within the PVDC w/V&G system, during the last 3 months of storage at 37°C/90%RH (Figure 11) resulted in greater lipid oxidation.

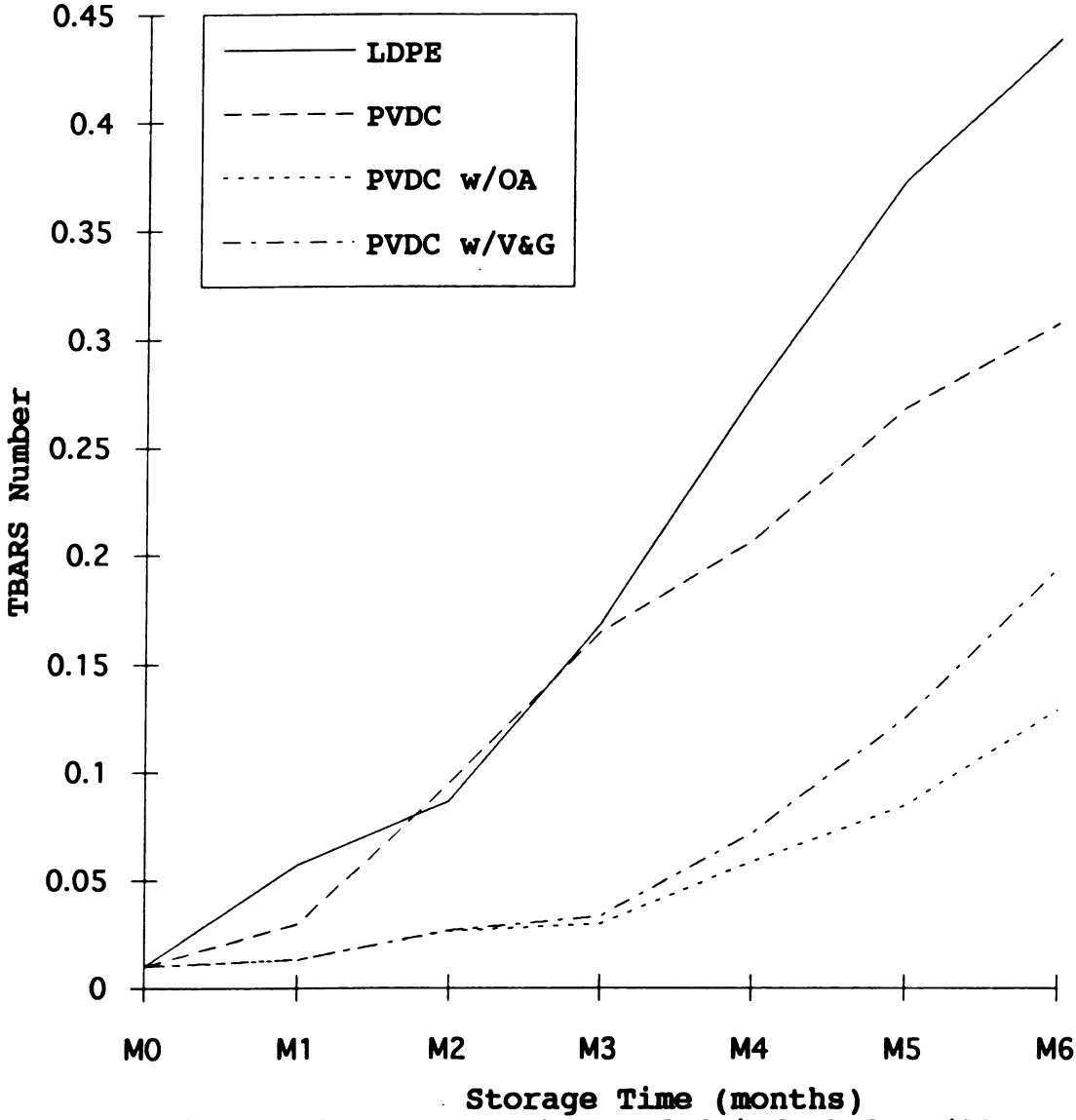


Figure 12: TBARS numbers of dried whole milk samples at 21° C/50%RH for 6 months

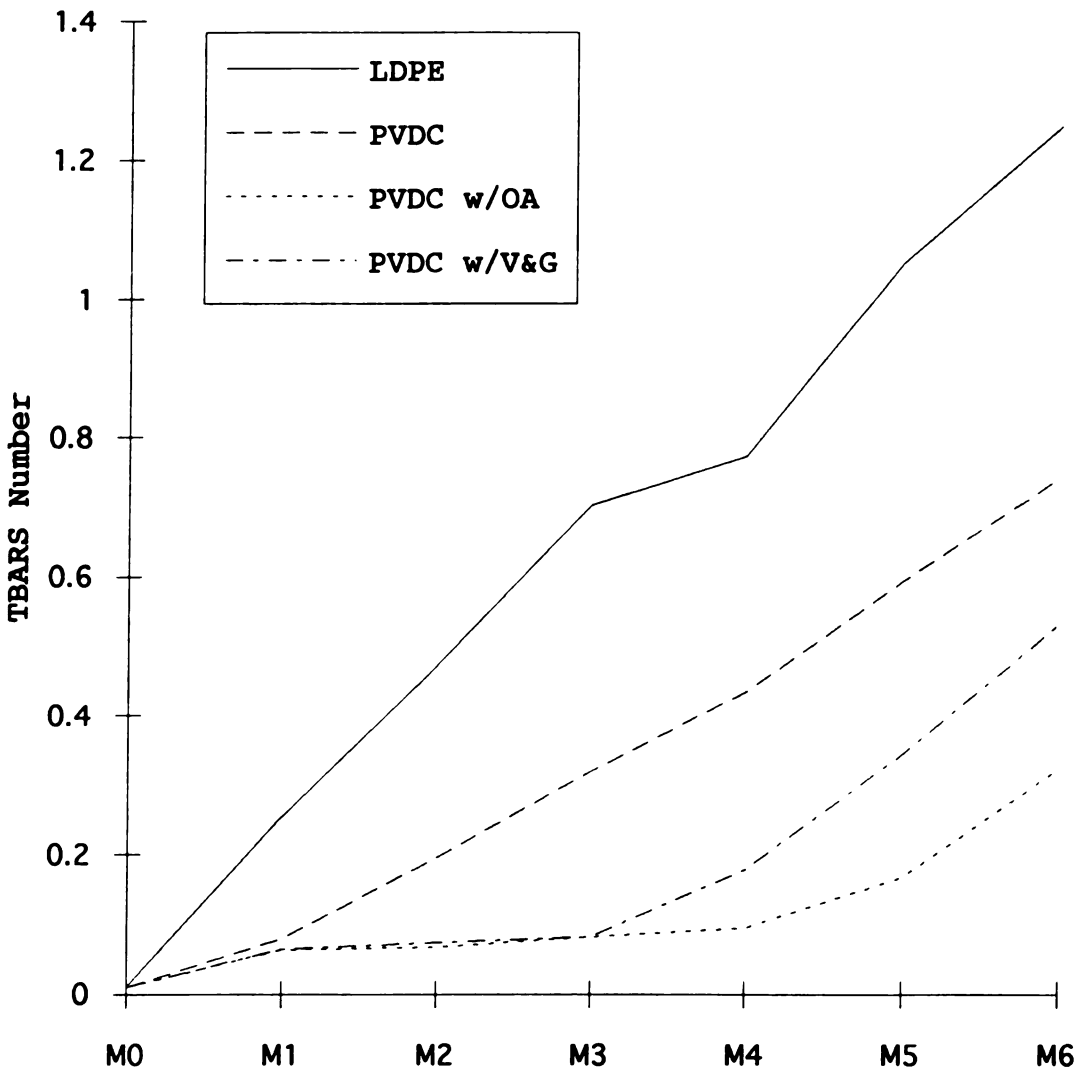


Figure 13: TBARS numbers of dried whole milk samples at 37° C/90%RH for 6 months

Oxidative changes in dried whole milk samples stored at 2 different storage conditions

The extent of lipid oxidation was significantly influenced by storage condition. In general, higher temperature accelerates the rate of the oxidative reaction. The TBARS numbers of each packaging system stored at 37°C/90%RH was distinctively higher than that of the same packaging system stored at 21°C/50%RH. For the PVDC w/OA system, however, there was the least difference in oxidation between products stored at 21°C/50%RH and 37°C/90%RH compared to other packaging systems (LDPE, PVDC, and PVDC w/V&G).

Likewise, high humidity storage can be detrimental to the oxidative stability of dried milk products (Hall and Hedrick, 1966). Therefore, the packaging material must provide a relatively high moisture barrier to protect dried milk products from moisture absorption during storage. From personal observation, dried milk samples packaged in LDPE pouches and stored at 37°C/90%RH showed the greater color change (light brown) after 10-weeks of storage. There was a linear correlation ($r = 0.90$) between moisture content and TBARS number of dried milk samples packaged in LDPE pouch and stored at 37°C/90%RH for 6 months. Correlation coefficient of dried whole milk samples packaged in PVDC pouches and stored at 37°C/90%RH for 6 months was $r = 0.78$, whereas, those of dried milk samples packaged in PVDC w/OA and PVDC w/V&G were low which indicated that higher moisture content in concert with sufficient oxygen elevates lipid oxidation.

Cholesterol oxidation in dried whole milk

The measurement of cholesterol oxides in dried whole milk requires the use of several delicate techniques. These include lipid extraction (Folch et al., 1957), cholesterol oxide cleanup procedure on silica cartridges (Morgan and Armstrong, 1989), derivatization to form trimethylsilyl ether derivatives, and quantification with capillary gas chromatography (GC). Cholesterol oxide products (COPs) in dried whole milk packaged in different packaging systems were quantified to evaluate cholesterol stability during storage.

Identification of COPs was based on the relative retention times of their trimethylsilyl ether derivatives, compared to those of cholesterol oxide standards. Using a standard mix, the response factor of each COP was calculated. Quantification of COPs was calculated by multiplying their response factors by the peak areas (see Appendix 3).

Since it is necessary to remove large amounts of triacylglycerols, cholesterol, and other nonpolar lipids from the total lipids and then concentrate trace amounts of cholesterol oxides prior to GC analysis, the fractional cleanup procedure (Nourooz-Zadeh and Appelqvist, 1988) on silica cartridges was applied. A response factor (RF) for each COP was calculated as described in Appendix 3. The amount of each COP, therefore, was corrected based on its relationship to the internal standard (6-ketocholesterol). Using silica column cleanup and an internal standard, Park

and Addis (1985a) reported nearly 100% recoveries of 7-ketocholesterol, 7α- and 7ß-hydroxycholesterols from beef. Nourooz-Zadeh and Appelqvist (1988) reported that this technique is practical and useful.

However, without skill and caution, some cholesterol oxides can be removed with the washing solutions, resulting in lower levels of COPs. On the other hand, over estimation of COPs in dried whole milk is possible. Heat, light, and air exposure during analysis can promote higher cholesterol oxides concentrations. In addition, cholesterol can be degraded to form some artifacts (Smith, 1981). Therefore, the milk samples were protected from exposure to heat, light, and air during analytical process.

To determine COPs in dried whole milk, milk fat was extracted from the samples. The total lipid contents ranged from 25.09 to 26.07%. Nourooz-Zadeh and Appelqvist (1988) reported mean values of total fat in whole milk powder at 24.0% (240 mg/g powder) as measured according to the standard International Dairy Federation (IDF) method.

In this study, it was found that 7-ketocholesterol was predominantly formed over other COPs. After 6-month storage at high temperature, many COPs were found in the dried milk samples packaged in the four packaging systems (Figure 17). The major COPs were 7-ketocholesterol, 7α-hydroxycholesterol, α-epoxide, and ß-epoxide (Figure 18). This is similar to what was found by Nourooz-Zadeh and Appelqvist (1988). 25-hydroxycholesterol are regarded as the

most atherogenic sterol oxides (Peng and Morin, 1991) and were present at very low levels (about 3.3 μg/g lipid) in the PVDC w/OA and PVDC w/V&G packaging systems even stored at high temperature for 6 months (Table 7).

The ratio of α-epoxide to ß-epoxide in the dried whole milk stored at 37°C/90%RH for 6 months was approximately 1.1:1 (LDPE pouches) and 1.2:1 (PVDC pouches). This is not consistent with the findings of Nourooz-Zadeh and Appelqvist (1988) who analyzed cholesterol oxides in 12 month old spray-dried whole milk packaged in closed paper cans at room temperature. They found higher ß-epoxide levels in a ratio of 1.3-2.6:1 depending on heating temperature during manufacturing.

The interesting point is that ß-epoxide levels were higher than α-epoxide levels in the dried whole milk packed in PVDC w/OA and PVDC w/V&G, when stored at high temperature for 6 months (Table 7). This is in contrast to the findings in LDPE and PVDC (without OA) where α-epoxide levels were higher than ß-epoxide levels. From the literature, the levels of cholesteryl α-epoxide and cholesteryl ß-epoxide in dried whole milk were mixed. The α/ß epoxide ratio of the milk samples changed greatly depending on storage time (Nourooz-Zadeh and Appelqvist, 1988).

Differences in composition, processing, and storage conditions account for differences in COPs levels (Sander et al., 1989). In addition, some COPs may be selectively washed out during cleanup to a greater degree than are 7-

ketocholesterol and the internal standard (Morgan and Armstrong, 1989). Certain COPs may also be gradually reduced or degraded during storage (Addis and Park, 1991).

Effects of packaging and storage condition on cholesterol stability

Initially, fresh dried whole milk was analyzed for COPs, but none was detected. Dried whole milk samples packaged in LDPE pouches and stored at 21°C/50%RH had total amounts of COPs from 1.75 (at 3rd month) to 7.99 (at 6th month) μg/g lipid (Table 6 and 7). According to Kim and Nawar (1992), the milk fat globule membrane (MFGM) has its own protective mechanism which protects cholesterol against oxidation. They reported that the nonlipid fraction protected cholesterol against oxidation, while the lipid fraction was destructive. Nevertheless, in the dry state, the net result between these opposing interactions was negative.

Table 6 : Cholesterol oxide concentrations in dried whole milk samples at 2 storage conditions for 3 months

Storage Cond	Pkg. System	COPs (μg/g lipid)							
		7-keto	7α-OH	7β-OH	α-epox	β-epox	20α-OH	25-OH	total
21℃/50%RH	LDPE	1.75	ND	ND	ND	ND	ND	ND	1.75
	PVDC	ND	ND	ND	ND	ND	ND	ND	0.00
	PVDC w/OA	ND	ND	ND	ND	ND	ND	ND	0.00
	PVDC w/V&G	ND	ND	ND	ND	ND	ND	ND	0.00
37℃/90%RH	LDPE	9.97	3.49	4.09	ND	ND	ND	2.18	19.73
	PVDC	1.97	1.67	ND	ND	ND	ND	ND	3.64
	PVDC w/OA	ND	ND	ND	ND	ND	ND	ND	0.00
	PVDC w/V&G	ND	ND	ND	ND	ND	ND	ND	0.00

Note : ND refers to not detected, detection limit 0.1 μg

Table 7: Cholesterol oxide concentrations in dried whole milk samples at 2 storage conditions for 6 months

Storage Cond	Pkg. System	COPs (μg/g lipid)							
		7-keto	7α-OH	7β-OH	α-epox.	β-epox.	20α-OH	25-OH	total
21℃/50%RH	LDPE	5.87	ND	2.12	ND	ND	ND	ND	7.99
	PVDC	2.88	ND	0.93	ND	ND	ND	ND	3.81
	PVDC w/OA	ND	ND	ND	ND	ND	ND	ND	0.00
	PVDC w/V&G	ND	ND	ND	ND	ND	ND	ND	0.00
37℃/90%RH	LDPE	42.97	49.44	13.19	46.97	42.29	19.57	32.31	246.74
	PVDC	26.15	3.26	10.84	9.62	7.95	3.79	9.90	71.51
	PVDC w/OA	19.69	2.59	4.43	7.76	11.33	ND	3.30	49.10
	PVDC w/V&G	9.71	5.77	6.83	2.99	3.34	ND	3.31	31.95

Note : ND refers to not detected, detection limit 0.1 μg

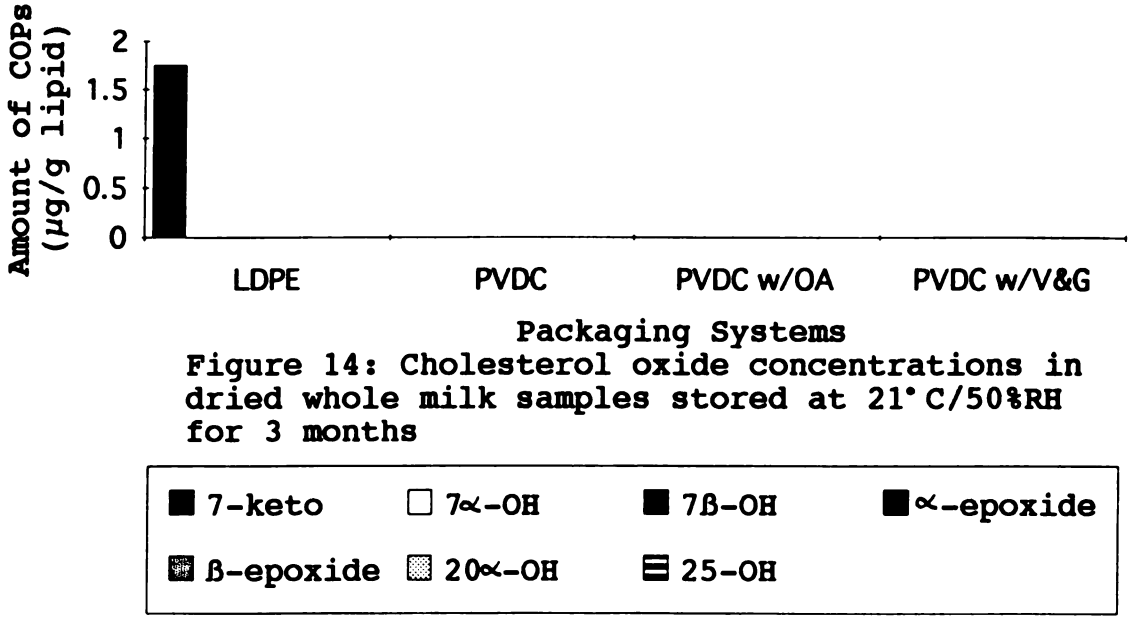


Figure 14: Cholesterol oxide concentrations in dried whole milk samples stored at 21°C/50%RH for 3 months

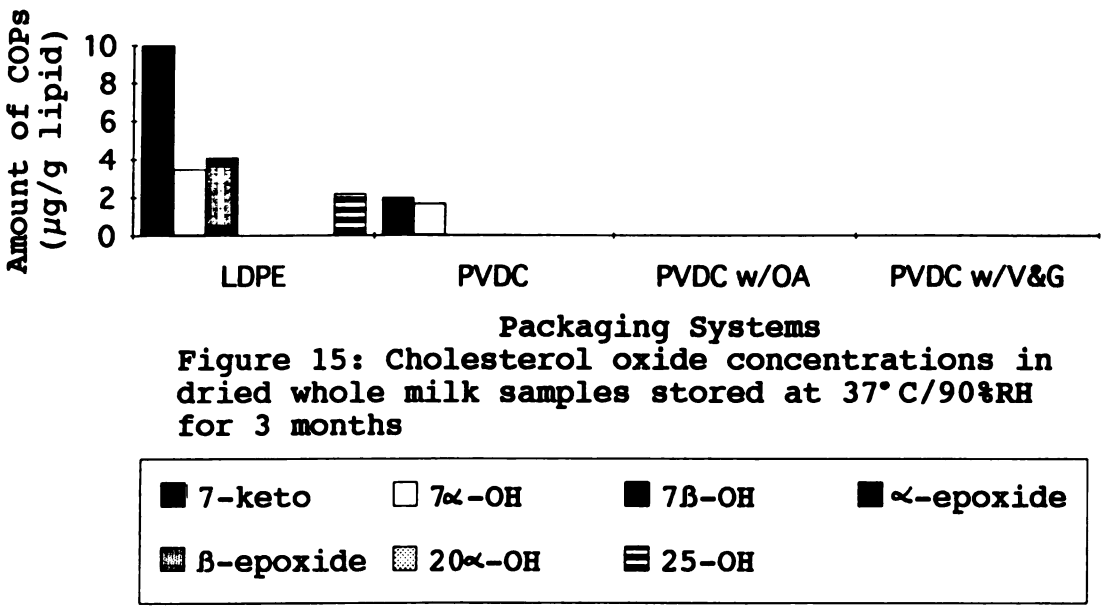


Figure 15: Cholesterol oxide concentrations in dried whole milk samples stored at 37°C/90%RH for 3 months

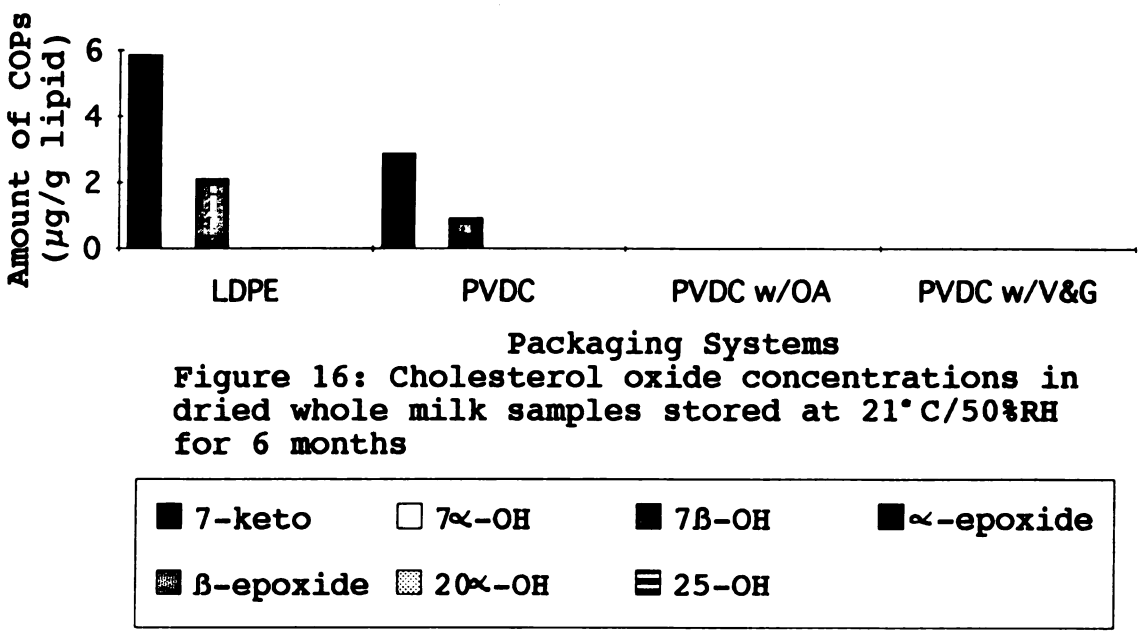


Figure 16: Cholesterol oxide concentrations in dried whole milk samples stored at 21°C/50%RH for 6 months

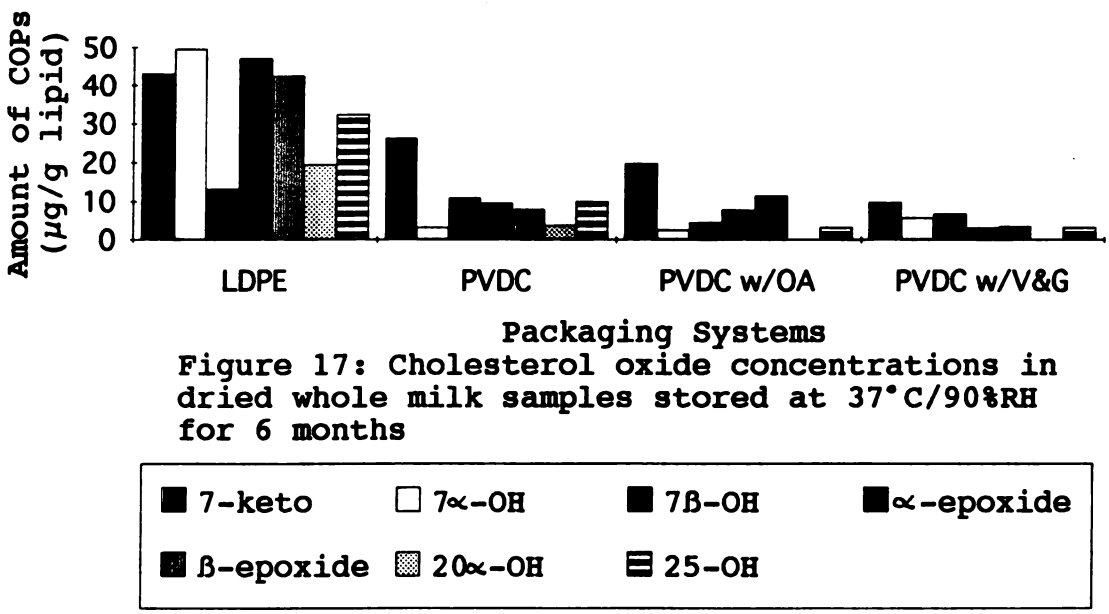


Figure 17: Cholesterol oxide concentrations in dried whole milk samples stored at 37°C/90%RH for 6 months

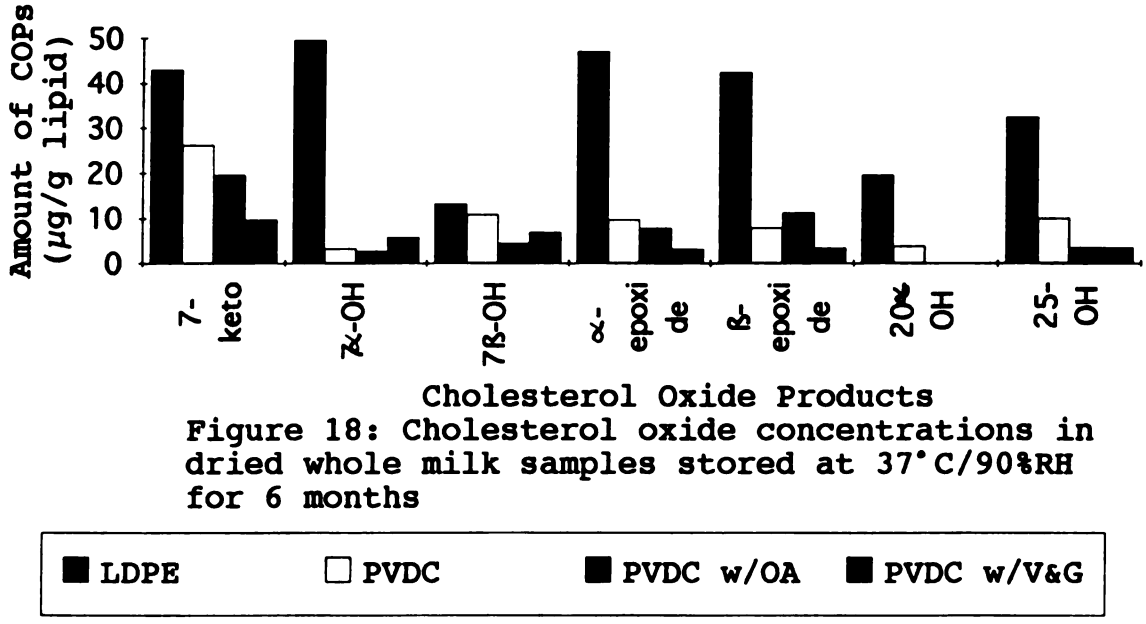


Figure 18: Cholesterol oxide concentrations in dried whole milk samples stored at 37°C/90%RH for 6 months

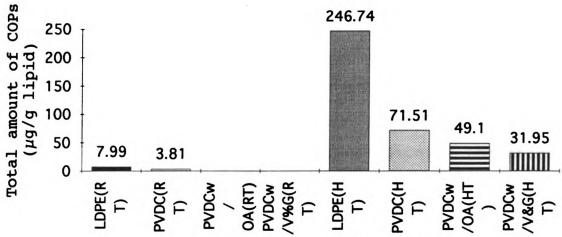


Figure 19: Total amounts of COPs in dried whole milk samples stored at 21°C/50%RH (RT) and 37°C/90%RH (HT) for 6 months

The total amount of COPs in LDPE pouched product stored at 37°C/90%RH for 6 months was 246.74 μg/g lipid, while at 21°C/50%RH, it was only 7.99 μg/g lipid (Figure 19). Therefore, exposure to high temperature during storage causes highly significant increases in total amounts of COPs. However, high temperature storage did not result in the same increase in cholesterol oxidation of the samples packaged in PVDC w/OA and PVDC w/V&G. No quantifiable COPs were found in the samples packaged in PVDC w/OA and PVDC w/V&G when stored at both ambient and high temperature for 3 months. When samples were stored for 6 months at ambient temperature, no COPs were found in these 2 packaging systems. The PVDC w/OA and PVDC w/V&G packaging systems were highly effective in protecting dried whole milk against cholesterol oxidation when stored at ambient temperature, due to the reduced oxygen content within the package.

Combining the destructive effects of high temperature and long storage time, cholesterol oxides were formed in samples packaged in PVDC w/OA and PVDC w/V&G. Total amount of COPs found in samples packaged in PVDC w/OA (49.10 μg/g lipid) was slightly higher than that found in PVDC w/V&G (31.95 μg/g lipid), when stored at 37°C/90%RH for 6 months. This may be due to degradation or reduction of some COPs during storage and/or mishandling during analytical procedure. However, there was a significant difference ($P=0.01$) between levels of COPs in these 2 packaging systems.

The highest total amount of COPs (246.74 μg/g lipid) was found in samples packaged in LDPE pouches and stored at 37°C/90%RH for 6 months. From the result of Chan (1992), the highest total amount of COPs, found in milk powder packed in PE pouches and stored at 40°C for 6 months, was 539.8 μg/g lipid. Formation of cholesterol oxides was greatly influenced by high temperature and long storage time, especially in the absence of an effective oxygen barrier. These factors synergistically promote cholesterol oxidation and resulted in the tremendous increase in cholesterol oxides in dried whole milk packaged in LDPE pouches.

Relationship between TBARS number and total cholesterol oxides concentration

Correlation coefficients between TBARS numbers and total cholesterol oxide concentrations in dried milk samples are shown in Table 8. TBARS numbers increased in a positive linear manner as total cholesterol oxide concentrations increased. Correlation coefficients ranged from 0.854 to 0.992 which indicates that TBARS number and total cholesterol oxide concentration were strongly correlated to each other. This indicates that extent of cholesterol oxidation during storage can be predicted by TBARS number.

Formation of cholesterol oxide products in dried whole milk is greatly influenced by storage condition and storage time. Generally, high temperatures accelerate the oxidation of cholesterol. Since oxygen is the major cause of

cholesterol oxidation, elimination of headspace oxygen within the package helps to prevent cholesterol oxidation in dried milk products. Reduced oxygen atmosphere packaging, such as oxygen absorbers and vacuum/gas flush packaging, helps maintain cholesterol stability.

Table 8: The relationship between TBARS numbers and total cholesterol oxide concentrations in dried whole milk samples packaged in different packaging systems for 6 months

Storage Cond.	Pkg. System	Best fit regression line[a]	Correlation coefficient(r)
21°C/50%RH	LDPE	Y = -0.68 + 19.09X	0.987
	PVDC	Y = -0.76 + 12.65X	0.854
	PVDC w/OA[b]	Y = 0X	-
	PVDC w/V&G[c]	Y = 0X	-
37°C/90%RH	LDPE	Y = -36.50 + 191.40X	0.868
	PVDC	Y = -11.16 + 101.40X	0.925
	PVDC w/OA	Y = -6.99 + 169.04X	0.975
	PVDC w/V&G	Y = -2.81 + 64.77X	0.992

[a]When X = TBARS number, Y = total cholesterol oxide concentrations

[b,c]Since no quantifiable COPs were detected in their dried milk samples, correlation coefficients are undefined.

SUMMARY AND CONCLUSIONS

Reduced oxygen atmosphere packaging with oxygen absorbers effectively protected lipid and cholesterol components of dried whole milk, thus, improving its long term quality. When 5 lb of dried whole milk is packaged with oxygen absorbers in a barrier package and stored at ambient condition (21°C/50%RH) no COPs were detectable in the powder. In addition, product packaged in this system (bulk package) had the least lipid oxidation (lowest TBARS number) when compared with samples packaged in the other packaging systems (LDPE pouch, PVDC pouch, and PVDC with vacuum/gas flushing). Oxygen absorbers effectively worked with bulk packaging of dried whole milk.

Oxygen absorbers effectively absorb headspace oxygen at a higher rate when stored at high temperature and high relative humidity (37°C/90%RH), compared with ambient conditions, because high temperature accelerates the oxygen absorbing reaction. However, dried whole milk samples packaged in PVDC w/OA and stored at 37°C/90%RH showed greater lipid oxidation (higher TBARS numbers) than those samples stored at 21°C/50%RH due to the higher temperature and relative humidity. PVDC protects against lipid oxidation better than LDPE due to its greater barrier against oxygen and moisture.

High temperature and high relative humidity greatly accelerated lipid and cholesterol oxidation in dried whole milk during storage. Reduced oxygen atmosphere packaging with oxygen absorber or vacuum/gas flushing effectively prevented oxidative deterioration.

There was a significant difference (P=0.05) in TBARS number (extent of lipid oxidation) between packaging with oxygen absorbers and vacuum/gas flush packaging, when stored at ambient condition. However, there were no COPs found in samples packaged in these 2 packaging systems when stored at the same condition (21°C/50%RH) for 6 months.

Compared with vacuum/gas flush packaging, applying oxygen absorbers to the packaging of dried whole milk is more beneficial because; (1) the nature of dried whole milk is difficult to vacuum and remove oxygen headspace to near zero, (2) applying oxygen absorbers needs only an in-line automatic insertion machine which is more easily done than vacuum/gas flush packaging, (3) oxygen absorbers can absorb oxygen which permeates through the packaging materials during storage.

With a larger size bulk package of dried whole milk such as a 50 lb bag, oxygen absorbers should be applied in combination with vacuum/gas flush packaging. The appropriate size of oxygen absorbers to absorb the residual oxygen in the headspace should be considered to maintain very low levels of headspace oxygen in the package during storage time. Dried milk products need highly protective packaging systems to

prevent them from oxidative changes as these products are used in the manufacture of various processed foods.

APPENDICES

APPENDIX 1

Reagents used for measurement of lipid oxidation

1. 12N HCl/H_2O, 1:2, v/v
2. Aqueous TBA solution, freshly prepared by dissolving 0.5766g of 2-thiobarbituric acid (TBA)(Sigma Chemical Co., Louis, MO) in 200 ml deionized water
3. 0.5% PG/EDTA solution, freshly prepared by mixing these two solutions; (a) 0.5 g of propyl gallate (PG) (Sigma Chemical Co., Louis, MO) in 10 ml of ethanol; (b) 0.5 g of ethylenediamime tetraacetic acid (EDTA) (J.T. Baker Inc., Phillipsburg, NJ) in 90 ml of deionized water

Reagents used for COPs analysis

1. Acetone, analytical grade (Mallinckrodt Specialty Chemical Co.,Paris, KY)
2. Diethy ether, analytical grade (J.T. Baker Inc., Phillipsburg, NJ)
3. Hexane, analytical grade (J.T. Baker Inc., Phillipsburg, NJ)
4. Hexane/diethyl ether, 95:5, v/v
5. Hexane/diethyl ether, 90:10, v/v
6. Hexane/diethyl ether, 80:20, v/v
7. Pyridine, silylation grade

8. BSTFA[N,OBis(trimethylsilyl)trifluoroacetamide] with 1% TMCS (trimethylchorosilane)

Pyridine and BSTFA were purchased from Pierce Chemical Co.(Rockford, IL)

APPENDIX 2

Extraction of lipids

Reagents used for extraction of lipids

1. Chloroform, analytical grade
   (J.T. Baker Inc., Phillipsburg, NJ)
2. Methanol, analytical grade
   (J.T. Baker Inc., Phillipsburg, NJ)
3. Chloroform/methanol, 2:1, v/v
4. Chloroform/methanol/water, 3:48:47, by volume
   (upper phase solution)
5. Anhydrous sodium sulfate (Na_2SO_4)
   (Mallinckrodt Specialty Chemicals Co.,
   Paris, KY)

Procedure

The method of Folch et al.(1957) was employed for lipid extraction of dried whole milk samples. Dried whole milk samples (2.00 g) were homogenized in 15 ml methanol with a Ultra-Turrax type homogenizer (Tekmar Co., Cincinnati, OH) for 1 minute. 30 ml of chloroform were added, and the homogenization was continued for 2 minutes. The homogenate was filtered through Whatman no. 1 filter paper. The milk residue was washed with 2 x 15 ml of chloroform/methanol (2:1, v/v). The filtrate portion was mixed with 10 ml deionized water and shaken for 30 seconds.

During centrifugation at 1000 x g for 10 minutes, the mixture separated into two phases. The upper phase was removed by siphoning. The interface and the inside of the tube wall were rinsed with 3 x 1.5 ml of upper phase solution (chloroform/methanol/water, 3:48:47, by volume), and the rinsing solution was completely removed by siphoning. The lower phase was poured through Whatman no. 1 filter paper containing anhydrous Na_2SO_4 to remove water, and evaporated under vacuum with a rotary evaporator at 30-35°C. The final lipid extract was redissolved in 5 ml hexane, in preparation for COPs analysis. If the lipid extract was not analyzed that day, it was stored under nitrogen gas at -20°C.

APPENDIX 3

Calculation for amount of COPs

Absolute response factors

An absolute response factor [absRF(i)] for each cholesterol oxide component (i) was calculated by dividing the amount (μg) of a component [W(i)], by the area of its peak in the analysis of a standard mix.

$$absRF(i) = W(i)/A(i)$$

An absolute response factor of internal standard [absRF'(IS)] from the analysis of sample was calculated by dividing the added amount of internal standard (6-ketocholesterol) by the area of its peak.

$$absRF'(IS) = W'(IS)/A'(IS)$$

Response factors

A response factor (RF) of each cholesterol oxide component (i) was calculated by multiplying its absolute response factor [absRF(i)] with the ratio recovery of internal standard [absRF'(IS)/absRF(IS)]

$$RF(i) = absRF(i) \times [absRF'(IS)/absRF(IS)]$$

Amounts of cholesterol oxide components

The amount (μg) of each cholesterol oxide component [Amt(i)] was calculated by multiplying the area peak of that cholesterol oxide [A(i)] with its response factor [RF(i)].

$$Amt(i) = RF(i) \times A(i)$$

The amount of each cholesterol oxide product (μg) was reported based on 1 gram of milk lipid.

LIST OF REFERENCES

REFERENCES

Abe, Y. 1991. Oxygen absorbers: Is it the answer to shelf life problems? Asia Pac. Food Ind. 3(5):66.

Abe, Y. and Kondoh, Y. 1989. Oxygen absorbers. In "Controlled/ Modified Atmosphere/Vacuum Packaging of Foods," ed. A. L. Brody, pp.149-158, Food & Nutrition Press, Inc., Connecticut.

Addis, P. B. 1986. Occurrence of lipid oxidation products in foods. Food Chem. Toxic. 24:1021.

Addis, P. B. and Park, S. W. 1991. Cholesterol oxide content of foods. In "Biological Effects of Cholesterol Oxides," ed. S. K. Peng and R. J. Morin, pp.71-88, CRC Press, Inc., Florida.

Anderson, M. 1981. Inhibition of lipolysis in bovine milk by proteose peptone. J. Dairy Res. 48:247.

Aurand, L. W., Boone, N. H., and Giddings, G. G. 1977. Superoxide and singlet oxygen in milk lipid oxidation. J. Dairy Sci. 60:363.

Baumgartner, W. A., Baker, N., Hill, V. A., and Wright, E. T. 1975. Novel interface in thiobarbituric acid assay for lipid peroxidation. Lipids. 10:309.

Belitz, H. D. and Grosch, W. 1987. Milk and dairy products. In "Food Chemistry," trans. D. Hadziyev, pp.377-391, Springer Verlag, New York.

Berlin, E. and Pallansch, M. J. 1963. Influence of drying methods on density and porosity of milk powder granules. J. Dairy Sci. 46:8.

Bernstein, B. A. 1978. Cytochromes in the fat globule membrane of bovine milk and in the microsomes of the lactating bovine mammary gland. Ph.D. Thesis, University of Wisconsin, Madison.

Bischoff, F. 1969. Carcinogenic effect of steroids. In "Advances in Lipid Research," ed. R. Paoletti and D. Kritchevsky. Academic Press, New York.

Boon, P. M., Keen, A. R., and Walker, N. J. 1976. Off-flavor in stored whole milk powder II. Separation and identification of individual monocarbonyl components. N. Z. J. Dairy Sci. Technol.11:189.

Bracco, U., Dalgo, J. H., and Bohren, H. 1972. Lipid composition of the fat soluble membrane of human and bovine milk. J. Dairy Sci. 55:65.

Carlsson, D. J., Suprunchuk, T., and Wiles, D. M. 1976. Photooxidation of unsaturated oils : Effects of singlet oxygen quenchers. J. Am. Oil Chem. Soc. 53:656.

Carnevale, S. 1988. Use of oxygen scavengers in wine packaging. In Paper presentedat Pack Alimentaire'88 - 2nd Annual Food and DrinkPackaging Conference, held March 22-24, 1988. at San Francisco, CA.

Chan, H. W. S. 1987. "Autoxidation of Unsaturated Lipids," Academic Press Inc., Orlando, FL.

Chan, S. 1992. Cholesterol oxidation in whole milk powder as influenced by processing and packaging. Master Thesis, Michigan State University, E. Lansing, MI.

Chen, Z. Y. and Nawar, W. W. 1991. Prooxidative and antioxidative effects of phospholipids on milk fat. J. Am. Oil Chem. Soc. 68:938.

Christie, W. W. 1983. The composition and structure of milk lipids. In "Developments in Dairy Chemistry," vol.2 - Lipids, ed. P. F. Fox, pp.1-35, Applied Science Publishers, New York.

Clough, R. L., Yee, B. G., and Foote, C. S. 1979. Chemistry of singlet oxygen : The unstable primary products of tocopherol photooxidation. J. Am. Oil Chem. Soc. 101:683.

Crackel, R. L., Gray, J. I., Pearson, A. M., Booren, A. M., and Buckley, D. J. 1988. Some further observations on the TBA test as an index of lipid oxidation in meats. Food Chem. 28:187.

Deeth, H. C. and Fitz-Gerald, C. H. 1983. Lipolytic enzymes and hydrolytic rancidity in milk and milk products. In "Developments in Dairy Chemistry," vol.2 - Lipids, ed. P. F. Fox, pp.195-239, Applied Science Publishers, New York.

Dugan, Jr., L. R. 1955. Stability and rancidity. J. Am. Oil Chem. Soc. 32:605.

Eskin, N. A. M. 1990. Biochemical changes in raw foods: Milk. In "Biochemistry of Foods," 2nd ed., pp.205-236, Acadamic Press, Inc., New York.

Flanagan, V. P., Ferretti, A., Schwartz, D. P., and Ruth, J. M. 1975. Characterization of two steroidal ketones and two isoprenoid alcohols in dairy products. J. Lipid Res. 16:97.

Findley, I. D., Smith, J. A. B., and Lea, C. H. 1945. Experiments on the use of antioxidant in spray-dried whole milk powder. J. Dairy Res. 14:165.

Finocchiaro, E. T., Lee, K., and Richardson, T. 1984. Identification and quantification of cholesterol oxides in grated chesse and bleached butter oil. J. Am. Oil Chem. Soc. 61:877.

Finocchiaro, E. T. and Richardson, T. 1983. Sterol oxides in foodstuffs : A review. J. Food Prot. 46:917.

Folch, J., Lees, M., and Sloane Stanley, G. H. 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226:497.

Frankel, E. N. 1984. Lipid oxidation : Mechanism, Products and biological significance. J. Am. Oil Chem. Soc. 61:1908.

Frankel, E. N. 1991. Review : Recent advences in lipid oxidation. J. Sci. Food Agric. 54:495.

Garton, G. A. 1964. "Metabolism and Physiological Significance of Lipids," ed. R. M. C. Dawson and D. N. Rhodes, pp.335, Wiley, New York.

Gray, J. I. 1978. Measurement of lipid oxidation : A review. J. Am. Oil Chem. Soc. 55:539.

Gray, J. I. and Monahan, F. J. 1992. Measurement of lipid oxidation in meat and meat products. Trends Food Sci. Technol. 3:315.

Greene, B. E. and Cumuze, T. H. 1981. Relationship between TBA numbers and inexperienced panelists' assessments of oxidized flavor in cooked beef. J. Food Sci. 47:52.

Gutteridge, J. M. C. and Halliwell, B. 1990. The measurement and mechanism of lipid peroxidation in biological systems. TIBS 15:129.

Hall, C. W. and Hedrick, T. I. 1966. "Drying Milk and Milk Products," AVI Publishing Company, Inc., Westport, Connecticut.

Hara, A. and Radin, S. N. 1978. Lipid extraction of tissues with a low toxicity solvent. Anal. Biochem. 90:420.

Harima, Y. 1990. Free oxygen scavenging packaging. In "Food Packaging," ed. Takashi Kadoya, pp.229-252, Academic Press, Inc., New York.

Higley, N. A., Taylor, S. L., Herian, A. M., and Lee, K. 1986. Cholesterol oxides in processed meats. Meat Sci. 16:175.

Hill, R. D., Leeuwen, V. V., and Wilkinson, R. A. 1977. Some factors influencing the autooxidation of milks rich in linoleic acid. N. Z. J. Dairy Sci. Technol. 12:69.

Idol, R. C. 1991. A critical review of in-package oxygen scavengers and moisture absorbers. In Paper presented at 6th International Conference on Controlled, Modified Atmosphere, Vacuum Packaging,CAP'91, held Jan 9-11, 1991 at San Diego, CA.

Igene, J. O. and Pearson, A. M. 1979. Role of phospholipids and triglycerides in warmed-over flavor development in meat model systems. J. Food Sci. 44:1285.

Jarrett, W. D. 1979. A review of the important trace elements in dairy products. Aust. J. Dairy Tech. 34:28.

Jensen, R. G., Ferris, A. M., Lammi-Keefe, C. J., and Henderson, R.A. 1990. Dairy Foods - Lipids of bovine and human milks: A comparison. J. Dairy Sci. 73:223.

Khor, H. T. and Chan, S. L. 1985. Comparative studies of three solvent mixtures for the extraction of soybean lipids. J. Am. Oil Chem. Soc. 62:98.

Kim, S. K. and Nawar, W. W. 1992. Oxidative interactions of cholesterol in the milk fat globule membrane. Lipids. 27:928.

King, J. 1955. Catalytic removal of oxygen from food containers. Food Mfg. 30:441.

Kondoh, Y. 1991. A Japanese perspective on oxygen scavengers. In Paper presented at 6th International Conference on Controlled, Modified Atmosphere, Vacuum Packaging, CAP'91, held Jan 9-11, 1991 at San Diego, CA.

Korycka-Dahl, M. and Richardson, T. 1980. Symposium: Oxidative changes in milk. J. Dairy Sci. 63:1181.

Kurtz, F. E., Tamsma, A., and Pallansch, M. J. 1970. Organoleptic properties of foam spray-dried products made from deodorized milkfat and skim milk. J. Dairy Sci. 54:173.

Kurtz, F. E., Tamsma, A., Selman, R. L., and Pallansch, M. J. 1969. Effect of pollution of air with ozone on flavor of spray-dried milks. J. Dairy Sci. 52:158.

Lindquist, K. and Brunner, J. R. 1962. Composition of the free fat of spray-dried whole milk. J. Dairy Sci. 45:661.

Lindsay, R. C. 1985. Food additives. In "Food Chemistry," 2nd ed., revised and expanded, pp.643-644, Marcel Dekker, Inc., New York.

Louis, P. J. 1991. Longer shelf-life with oxygen absorbers. Eur. Packaging Newsl. World Rep. 24:1.

Luby, J. M., Gray, J. I., and Harte, B. R. 1986. Effects of packaging and light source on the oxidative stability of cholesterol in butter. J. Food Sci. 51:908.

Maeker, G. and Unruh, J. Jr. 1986. Cholesterol oxides I: Isolation and determination of some cholesterol oxidation products. J. Am. Oil Chem. Soc. 63:767.

Manning, P. B., Coulter, S. T., and Jenness, R. 1986. Determination of nitrate and nitrate and nitrite in milk and dry milk products. J. Dairy Sci. 51:1725.

Marcuse, R. and Johansson, L. 1973. Studies on the TBA test for rancidity grading 2. TBA reactivity of different aldehyde classes. J. Am. Oil Chem. Soc. 50:387.

Min, D. B., Lee, S. H., Lindamood, J. B., Chang, K. S., and Reineccius, G. A. 1989. Effects of packaging conditions on the flavor stability of dry whole milk. J. Food Sci. 54:1222.

Morgan, J. N. and Armstrong, D. J. 1989. Wide-bore capillary gas chromatographic method for quantification of cholesterol oxidation products in egg yolk powder. J. Food Sci. 54:427.

Naber, E. C. and Biggert, M. D. 1985. Analysis for the generation of cholesterol oxidation products in egg yolk by heat treatment. Poultry Sci. 64:341.

Nawar, W. W. 1985. Lipids. In "Food Chemistry," 2nd ed., revised and expanded, pp.198-205, Marcel Dekker, Inc., New York.

Nourooz-Zadeh, J. and Appelqvist, L. 1987. Cholesterol oxides in Swedish foods and food ingredients: Fresh eggs and dehydrated egg products. J. Food Sci. 52:57.

Nourooz-Zadeh, J. and Appelqvist, L. 1988. Cholesteroloxides in Swedish foods and food ingredients: Milk powder products. J. Food Sci. 53:74.

Park, S. W. and Addis, P. B. 1985a. HPLC determination of oxidized cholesterol derivates in foods. J. Food Sci. 50:1437.

Park, S. W. and Addis, P. B. 1985b. Capillary column gas-chormatographic resolution of oxidized cholesterol derivatives. Anal. Biochem. 149:275.

Park, S. W. and Addis, P. B. 1987. Cholesterol oxidation products in some muscle foods. J. Food Sci. 52:1500.

Park, S. W. and Addis, P. B. 1991. Methods of analysis of cholesterol oxides. In "Biological Effects of Cholesterol Oxides," ed. Shi-Kaung Peng and R. J. Morin, pp.33-70, CRC Press, Florida.

Patton, S. 1974. Malonaldehyde, lipid oxidation and thiobarbituric acid test. J. Am. Oil Chem. Soc. 51:114.

Patton, S. and Jensen, R. G. 1975. Lipid metabolism and membrane functions of the mammary gland. Prog. Chem. Fats Other Lipids. 14:167.

Patton, S. and Kurtz, G. W. 1951. 2-thiobarbituric acid as a reagent for detecting milkfat oxidation. J. Dairy Sci. 34:669.

Peng, S. K. and Morin, R. J. 1991. "Biological Effects of Cholesterol Oxides," CRC Press, Inc., Florida.

Peters, J. W. 1974. Packaging film improves shelf life, protects flavor by removing oxygen, halting oxidative changes. Food Product Development. 8(6):66.

Pomeranz, Y. and Meloan, C. E. 1987. Lipids. In "Food Analysis: Theory and Practice," 2nd ed., pp.691-748, Van Nostrand Reinhold Company, New York.

Powers, E. M. and Berkowitz, D. 1990. Efficacy of an oxygen scavenger to modify the atmosphere and prevent mold growth on meal, ready-to-eat pouched bread. J. Food Protection. 53:767.

Pryor, W. A., Stanley, J. P., and Blair, E. 1976. Autoxidation of polyunsaturated fatty acids II. A suggested mechanism for the formation of thiobarbituric acid-reactive materials from prostaglandin-like endoperoxides. Lipids. 11:370.

Rhee, K. S. 1978. Minimization of further lipid peroxidation in the meat distillation 2-thiobarbituric acid test of fish and meat. J. Food Sci. 43:1776.

Richardson, T. and Korycka-Dahl, M. 1983. Lipid oxidation. In "Developments in Dairy Chemistry," vol.2 - Lipids, ed. P. F. Fox, pp.241-363, Applied Science Publishers, New York.

Russell, C. E. and Gray, I. K. 1979. The cholesterol content of dairy products. N. Z. J. Dairy Sci. Technol. 14:281.

Sander, B. D., Addis, P. B., Park, S. W., and Smith, D. E. 1989. Quantification of cholesterol oxidation products in a variety of foods. J. Food Prot. 52:109.

Sander, B. D., Smith, D. E., and Addis, P. B. 1988. Effects of processing stage and storage conditions on cholesterol oxidation products in butter and cheddar cheese. J. Dairy Sci. 71:3173.

Sevanian, A. and Peterson, A. R. 1986. The cytotoxic and mutagenic properties of cholesterol oxidation products. Food Chem. Toxicol. 24:1103.

Sidwell, C. G., Salwin, H., and Mitchell, Jr., J. H. 1955. Measurement of oxidation in dried milk products with thiobarbituric acid. J. Am. Oil Chem. Soc. 32:13.

Sinnhuber, R. O., Yu, T. C., and Chang, Y. T. 1958. Characterization of the red pigment formed in the 2-thiobarbituric aciddetermination of oxidative rancidity. Food Res. 23:626.

Smith, L. L. 1981. "Cholesterol Autoxidation." Plenum Press, New York.

Smith, J. P., Oorailkul, W. J. K., Jackson, E. D., and Lawrence, R. A. 1986. Novel approach to oxygen control in modified atmosphere packaging of bakery products. Food Microbiol. 3:315.

Sommerfeldt, J. L. and Baer, R. J. 1986. Variability of milk components in 1705 herds. J. Food Prot. 49:729.

Spaulding, M. 1988. Oxygen absorbers keep food fresher. Packaging (US) 33:9.

Stansby, M. E. 1990. Deterioraton. In "Fish Oils in Nutrition," ed. M. E. Stansby, pp.120-140, Van Nostrand Reinhold, New York.

Swaisgood, H. E. 1985. Characteristics of edible fluids of animal origin : Milk. In "Food Chemistry," ed. O. R. Fennema, pp. 791-827, Marcel Dekker, Inc., New York.

Tamsma, A., Kurtz, F. E., and Pallansch, M. J. 1973. Comparison of flavor and physical properties of foam spray-dried whole milk prepared from concentrates foamed with air and with nitrogen. J. Dairy Sci. 56:161.

Tappel, A. L. 1962. Heme compounds and lipoxidase as biocatalysts. In "Symposium on Foods: Lipids and their Oxidation," ed. H. W. Schultz, E. A. D. and R. O. Sinnhuber, pp.122. AVI Publishing Company, Inc., Westport, Connecticut.

Tarladgis, B. G., Pearson, A. M., and Dugan, Jr., L. R. 1964. Chemistry of the 2-thiobarbituric acid test for determination of oxidative rancidity in foods II: Formation of the TBA-malonaldehyde complex without acid-heat treatment. J. Sci. Fd. Agric. 15:602.

Tarladgis, B. G., Watts, B. M., Younathan, M. T., and Dugan, L. R. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Am. Oil Chem. Soc. 37:44.

Te Whaiti, I. E. and Fryer, T. F. 1976. Factors influencing the release of free fat in raw milk. N. Z. J. Sci. Technol. 11:91.

Timmen, H. and Patton, S. 1988. Milk fat globules: Fatty acid composition, size and *in vivo* regulation of fat liquidity. Lipids 23:685.

Tsai, L. S. and Hudson, C. A. 1984. Cholesterol oxides in commercial dry egg products: Isolation and identification. J. Food Sci. 49:1245.

Tuohy, J. J. 1987. Milkfat autoxidation in whole milk powder during storage. Ph.D Dissertation, University of Ireland, Ireland.

Wade, V. N., Al-Tahiri, R., and Crawford, R. J. M. 1986. The autoxidation stability of annhydrous milk fat with and without antioxidants. Milchwissenschaft 41:479.

Warmbier, H. C. and Wolf, M. J. 1976. A pouch for oxygen-sensitive products. Modern Packaging 49(7):38.

Yuki, E. and Wadaka, H. 1972. Practical studies on the nitrogen gas exchange for preventing the oxidative rancidity of fatty food. J. Jpn. Food Sci. and Technol. 19:200.

Zenner, B. D. and Salame, M. 1989. A new oxygen absorbing system to extend the shelf-life of oxygen sensitive beverages. In Paperpresented at 13th International Ryder Conference on Beverage Packaging, BEV-PAK'89, held April 3-5, 1989, pp.205-220, Aquanautics Corp., Alameda, CA.

Zimmerman, P. L., Ernst, L. J., and Ossian, W. F. 1974. Scavenger pouch protects oxygen-sensitive foods. Food Technol. 28:63.