CHANGES IN CONCENTRATIONS OF SOME ALDEHYDES AFTER LIGHT EXPOSURE OR COPPER TREATMENT OF: A. MILK TREATED WITH SOME ANTIOXIDANTS, OR B. VARIOUS FRACTIONS OF MILK.

by

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INTRODUCTION

Light-induced off-flavors created by fluorescent display-lights in supermarkets have become a significant problem. Barnard (5) reported that in 1970, in Pennsylvania, 86.1% of the milk in all plastic containers had an oxidized flavor. According to Gregory et al. (25) nearly all of the all-plastic containers checked had some degree of light-induced and oxidized flavor. Thus, light-induced and oxidized flavors are wide spread, serious flavor defects. Although some consumers may become conditioned to the light-induced flavor, it is probable that it contributes to declining consumption of fluid milk.

Two distinct flavors develop in milk exposed to light (19, 27): first, a burnt-activated or "sunlight" flavor which develops rapidly due to the degradation of proteins and/or amino acids, and then a typical oxidized, papery card-boardy, cappy, metallic, tallowy, or oily flavor. The latter develops on prolonged exposure, due to the oxidation of milk lipids. Much attention has been given to the copper content of milk and dairy products because of its catalytic effect on the development of this lipid oxidized flavor. Milk from the processor may contain both natural and contaminent copper. The latter can gain entrance to milk during production, i.e., during milking, cooling, pumping, etc. Copper, however, is a normal component of milk, present in amounts of about 20 to 500 ug per liter (12, 13).

Lecithin, cephalin, and sphingomyelin, which are present in milk, are known collectively as phospholipids or phospha-

tides. They are fat like substances, containing phosphorus and nitrogen, associated with the protein of milk, and are easily oxidized. The total phospholipid content in whole milk has been reported to be 0.028, 0.031, 0.037% (54a). In fluid milk products, such as milk, cream, skim milk, and sweet-cream buttermilk, the substrate for oxidation is largely, if not entirely, phospholipid. When such products oxidize, the iodine values of phospholipids fall while those of triglycerides remain unchanged (44). Products of milk fat oxidation that have been most intensively investigated are the volatile carbonyl compounds (33). They are organoleptically (23, 37, 42) and instrumentally (6, 7, 9, 10) detectable at concentrations of parts per billion in liquid milk.

REVIEW OF LITERATURE

I. Milk Flavor.

A. The natural flavor of milk.

Milk produced under proper conditions has a mild, pleasant flavor. Any detectable aroma and flavor is classed as abnormal or off-flavor.

B. Off-flavors related to fluid milk.

The present terminology for describing off-flavors in milk consists of a mixture of types of terms. Descriptive terms such as papery, associative terms such as oily, or causative terms such as oxidized are used. Regardless of which term is used, the remedy lies in preventing oxidation. It is recognized that there may be cases where the cause of a particular flavor is unknown.

Causes of off-flavors were divided into seven categories which are listed in the following table along with descriptive and associative terms.

*Categories of Off-Flavors in Milk

Causes	Descriptive or Associative Terms
1. Heated	Cooked, carmelized, schorched
2. Light-induced	light, sunlight, activated
3. Lipolyzed	rancid, butyric, bitter, goaty
4. Microbial	acid, bitter, fruity, malty, putrid, unclean
5. Oxidized	papery, cardboard, metallic, oily, fishy
6. Transmitted	feed, weed, absorbed, cowy, barny
7. Miscellaneous	astringent, flat, foreign, medicinal, chalky, salty

*Prepared by the Committee on Flavor Nomenclature and Reference Standards.

Light-induced off-flavors. Milk exposed to various forms of radiant energy develops off-flavors. These are of practical importance when milk is exposed sufficiently to direct sunlight, fluorescent light or even diffused daylight. The activated or sunlight flavor develops rapidly and has been attributed to a degradation of some serum protein components; the other, a tallowy flavor, is attributed to lipid oxidation. The latter appears to develop more slowly. The lipid oxidation segment of the flavor may be similar to typical oxidized flavor from volatile carbonyl compounds. Bandler (4) found oxidized flavor to be the most serious off-flavor among 501 samples of bottle milk in New York during a survey in 1969. Barnard (5) pointed out that the term "oxidized" covers a broad range of related off-flavors, and some occur spontaneously or are caused by factors other than light exposure.

Several investigators (32, 51, 52) implicated methional from the degradation of milk proteins and/or amino acid methionine in the presence of riboflavin (vitamin B_2) under the active influence of light, particularly sunlight as the chief cause of the light-activated off-flavor (sunlight flavor).

Patton (40) and Patton and Josephson (41) were of the opinion that the casein acts as the chief source of methional as it is the milk protein substance richest in methionine while at the same time being opaque to light.

However, they presented no other proof in support of this contention. Storgards and Lindqvist (51) concluded from their studies that serum protein, although probably not the only factor, are more significant source of the substances causing the light-activated flavor than is the milk casein. They reported that light-induced flavor forms at least as rapidly in whey (de-fatted) as in skim milk. They also found these flavor-imparting substances were dialyzed through cellophane. Since dialysis takes place relatively slowly, the flavor-imparting substances must be relatively high molecular weight. They are absorbed by carbon and very soluble in fat. These properties coincide with those of free methional. Finally, they concluded that the oxidation of methionine appears to take place without first being split-off from the serum protein molecule and riboflavin seems to enhance light-induced flavor.

Wishner et al. (55) demonstrated that methional could not be detected in the form of its 2,4-dinitrophenylhydrazone in light exposed milk. They were able, however, to obtain a 20% recovery (in their analysis) of synthetic methional added to milk at the 1 ppm level. The flavor threshold of methional in milk has been determined to be in the order of 20-50 ppb (34). Ballance (3) observed that dry methional decomposes in the presence of ninhydrin to methyl mercaptan, dimethyl sulfide, and acrolein. Samuelsson (45) showed that methionine is converted by an apparently zero-order reaction to mercaptans, sulfides,

and disulfides at pH 6.8 in the presence of light and oxygen. He also reported that insulin, which contains no methionine, produces no sulfur-containing substance or odor upon irradiation. Since the flavor thresholds in water for dimethyl sulfide and methyl mercaptan are 12 and 2 ppb, respectively, Samuelsson believes that these compounds are responsible for the off-flavor. The previously indicated role of methional in sunlight milk flavor may be mediated by its conversion to other flavorful sulfur compounds.

In the dairy literature, there is agreement that: (a) oxidized and "sunlight" flavors are the two principal off-flavors induced by light; (b) the wavelengths of light inducing the off-flavor reactions are within the range of the visible spectrum; (c) absence of air retards off-flavor development; and (d) riboflavin is directly involved in the production of flavor defect.

Singleton et al. (50) reported that a maximum light activated flavor developed, and destruction of riboflavin and tryptophan occurred from a monochromatic light with a wavelength of 450 nm. They also reported that a milk sample exposed to sunlight developed light-induced flavor; whereas, it did not develop an oxidized flavor as measured by thiobarbituric acid (TBA) method (35). The TBA method is usually used to measure the development of lipid oxidized flavors. Although the intensity of sunlight flavor increased a lot from milk samples after light exposure, the TBA values

did not change much. According to this, they concluded that sunlight flavor is not a blend of oxidized and activated flavor, because the rate of oxidation of protein is rapid while that of lipids is relatively slow. Typical oxidized flavors developed on prolonged light exposure due to the autoxidation of milk lipids. The autoxidation results in a series of saturated and unsaturated aldehydes, ketones and possibly other carbonyl compounds due to the break down of hydroperoxides formed by chain reactions (2). Aurand et al. (1) showed that the oxidized flavor due to lipid oxidation was not detectable until a TBA value of 0.055. Dimick (16) and Bassette (6) reported that TBA test for fat oxidation was a poor measure of the volatile materials produced in light-activated milk and the TBA values did not parallel organoleptic properties of the milk.

Finley et al. (22) isolated a low density lipid-protein fraction from milk by the following procedures. (a) Milk samples were centrifuged in 40 ml centrifuge tubes in an International B-20 centrifuge at 42,000 x g at 4 C for two hours and the cream layer was removed. (b) The cream layer obtained was increased in volume to 40 ml with milk dialysate and centrifuged again at 30 C. Centrifugation yielded 5 fractions: fat, low density lipoprotein (LDLP), serum, high density lipoprotein and a heavy precipitate. The LDLP fraction from centrifugation was extracted 3 times with diethyl ether to remove neutral lipids. The 5 purified centrifugal fractions prepared from unexposed and

exposed milk were added to unexposed milk of the same origin. The organoleptical scores indicated that the light-induced off-flavor was in the LDLP fraction. They also indicated that the protein portion of the LDLP fraction appeared to undergo a partial degradation after light exposure resulting in losses of tryptophan, tyrosine, lysine, cysteine and methionine. The lipid portion was partially oxidized as indicated by decreased oleic and linoleic acid. Moreover, they proved by the thin layer chromatography (TLC) that light-induced changes in the lipid portion were different from those induced by addition of copper. According to Storgards and Lindqvist (51), the origin of the light-induced flavor is not always limited to the LDLP fractions.

Bassette in 1976 (6) found that exposing skim milk to sunlight for 20 min produced more acetal than by similarly treating homogenized-pasteurized milk indicating that non-fat fractions are precursors of acetal. He also demonstrated that n-pentanal increased more than n-hexanal in milk after 20 min sunlight exposure, while n-hexanal increased more than n-pentanal in milk after 5 ppm copper exposure for 23 hr.

Sattar et al. (48) in 1976 reported a decrease in oxidation rate (OR) by increasing the wavelength of light in milk samples. Short wavelengths (350-455 nm) were absorbed, whereas the samples were transparent to longer wavelengths (500-750 nm). The photo-oxidative effect/unit of radiant

energy intensity in relation to the wavelength varied characteristically for each oil or fat. OR, as determined in the dark, was greater in photo-bleached milk fat than in any other oil. The susceptibility of lipids to photo-oxidation did not depend on the degree of unsaturation alone; although there is a consistent idea that phospholipids which contain more unsaturated fatty acids than the triglyceride fraction in milk are responsible for lipid oxidation and the oxidized flavor (2, 24, 47).

Copper-induced oxidized flavor. El-Negoumy (20) pointed out that the natural and contaminated copper are detrimental to milk flavor. In fluid milk, the essentials of the lipid oxidation appear to be air, the proper redox potential and trace metal catalysis (copper particularly and iron much less effectively).

According to Samuelsson (46, 47), raw milk from a producer normally contains about 0.05 ppm copper, and only between 10 and 35% of this natural copper concentrates on the fat globule surfaces. They also reported that only 2-3% of the added contaminant copper was associated with the fat globules. While 9.8% remained in unwashed cream, 30% in casein or Na-caseinate, and 60% of the added copper was in whey proteins.

El-Negoumy et al. (20) concluded that the fat globule membrane is the main source of off-flavors resulting from lipid oxidation in aqueous dairy products, doubtlessly because of its higher concentration of phospholipids, in the

form of phospholipid-protein (lipoprotein) complex, and natural copper in the membrane as compared with the triglyceride fraction.

Samuelsson (47) also pointed out that a high sulfur content in milk is associated with a high copper content. The phospholipids are the first compounds to be oxidized in milk and dairy products. Added copper associates with these easily oxidizable substances and this might explain why contamination with copper so readily catalyzed development of the oxidized flavor. There are several reports (28, 30, 53) that the membrane proteins and whey proteins are relatively rich in sulfur.

C. Milk lipids, pasteurization (non-homogenized), homogenization and their relationship to oxidized flavor in fluid milk.

The fat globules in milk contain approximately 99% triglycerides with fatty acids containing carbon chain lengths between C_4 and C_{26} (2). According to Webb et al. (54b), the percentage of total phospholipids is 0.80-1.00% of the total milk lipids while 95-96% is triglycerides. They also mentioned that the fat globules derived their stability from the membrane which contains mainly a phospholipid-protein complex (lipoprotein). Since the surface layers of the globules are readily altered (by stirring, churning, foaming, etc.), the composition of the layers may change with the treatment to which the milk is subjected. Short chain fatty acids are not present in milk phospholipids

which contain more unsaturated fatty acids (oleic acid 40.3% and linoleic acid 6.1%). According to Kurtz (54c), about 50% of the phospholipids are found in skim milk, probably as components of small fat globules and lipoprotein particles; most of the phospholipids associated with cream are recovered in the aqueous phase (buttermilk) after churning. Koops et al. (36) also illustrated the migration of phosphatides during processing of dairy products. Presumably, lipoprotein particles are desorbed from the fat globule surface by physical agitation. Greenbank and Michael (26) reported that any form of agitation caused a migration of phosphatides from the globule membrane to the skim milk. Homogenization at or below 2,000 psi caused a migration of phosphatides away from the fat globule surface, but higher pressures reversed this migration until at a pressure of 8,000 psi the amount of phosphatides associated with the fat phase was approximately equal to that in the untreated milk. They also reported that there was 14.9% of the total phosphatides migrating from cream to skim milk at 63 C for 30 min of pasteurization, but only 5.5% migrating from cream to skim milk at 74 C for 15 s. In general, dairy literature suggests a relationship between unsaturated fatty acids (oleic and linoleic acids) and susceptibility to oxidized flavor in fluid milk.

Homogenization increases dispersion of lipid phase and its interfacial boundary surface (i.e., homogenization

reduces by 2 to 3 times the amount of phospholipids per unit of globular surface). This fact may explain the whiter color of homogenized milk itself, the increase in sensitivity to light-induced deterioration, and the inhibition of copper-induced oxidative changes (54d).

II. Evaluation of Antioxidants.

Dahle and Nelson in 1941 (14) extracted an antioxidative substance from oat and soybean flours with water at 57 C (8% of flour in final concentration) and with acetone, ethyl alcohol, ethyl ether, and hexane at 20 C (20% of flour in final concentration). They added 0.05% and 0.1% of each extract of antioxidative properties to dry, milk fat and showed that the peroxide numbers were lower after storage compared with those of control without adding any extract. They also reported that the relatively large amount of antioxidant may produce certain undesirable effects in the dairy product. Consequently, a more concentrated form of the antioxidative substance would be desirable.

Sidhu, Brown, and Johnson (49) in 1975 suggested that an antioxidant, in order to be successful, should check lipid oxidation in milk for at least 14 days, should not impart its own off-flavor to milk, and should be acceptable as a food additive. Of the antioxidants tested on linoleic acid rich milk, BHA, sesamol, BHA + propylgallate, BHA + γ-tocopherol, NDGA and ethoxyquin were all effective in controlling oxidized flavors for 14 days and imparted no detectable off-flavor of

their own. These antioxidants also reduced O_2 uptake more or less to the level observed in conventional milk after storage for 10 days. Tert-butylhydroquinone was effective, but imparted off-flavors. PG, GT-1 (γ-tocopherol), GT-2 (water dispersible γ-tocopherol), NF4-50 (α-tocopherol), ascorbyl palmitate, and antioxidant Roche D-20 (α-tocopherol + ascorbyl palmitate) and Tenox 2 were only partially effective. Antioxidant off-flavors showed a tendency to disappear or were masked by oxidized or other off-flavors developed subsequently during storage. They also concluded that of the 2 phenolic antioxidants, BHA was effective and BHT ineffective.

III. Methods of Analysis.

A. Isolation of the flavor fraction.

Methods for the isolation and concentration of volatile flavor compounds from food products usually consist of vacuum distillation at atmospheric or low pressure, fractional distillation, extraction, freeze concentration, and absorption. Day and Lillard (15) applied steam distillation at 3 mm Hg absolute pressure to isolate volatile carbonyl compounds in oxidized milk fat. 2,4-DNP-hydrazine was added and the DNP-hydrazones of the carbonyls once formed were extracted with hexane. A review dealing specifically with analytical techniques used with dairy products was published by Evans in 1972 (21).

Steam distillation generally effects the concentration of the flavor volatiles and the presence of water in the

distillate may present a problem in gas chromatography. Honkanen and Karvonen (31) demonstrated that, for high molecular weight compounds, distillation may not be the best method for isolating flavor components. Using GLC, they showed that in general, the larger the molecular sizes of the flavor compound, the lower their percent recovery from milk. On the other hand, they were able to obtain recoveries between 50% and 90% for compounds boiling at 100-250 C at concentrations of 0.005 to 10 ppm. Dimick and Walker (17) observed that at high distillation temperatures, certain precursor compounds in milk fat are completely converted to their off-flavor derivatives (e.g., lactones and methyl ketones). At low temperatures these reactions can be minimized or avoided, but then the off-flavored compounds in the fat are not completely removed. Badings (2) reported that by using a batch process high-vacuum distillation of butter fat to which alkanal C_5-C_{10} inclusive had been added in concentrations of 0.5 to 1 ppm. They recovered 83% in average of those compounds by GLC examination. Bassette and Ward (9, 10) combined rapid steam distillation and head space sampling for GLC in quantitative and qualitative analyses of part per million and parts per billion levels of volatile materials in biological fluids (e.g., urine, cow plasma, milk, etc.).

It is now well recognized that, while the even-numbered straight-chain fatty acids constitute the major components of natural fats, trace quantities of other fatty acids are

also present. Evidence was presented which demonstrated that the precursors of the C_5 through C_{15} odd-numbered methyl ketones were triglycerides containing one β-keto acid and two fatty acid moieties. These glycerides accounted for 0.045% of the butter fat. β-keto acids have been recognized as an additional class of trace constituent of the triglycerides of milk fat (29). A homologous series of methyl ketones with odd numbers of carbon atoms were found by Patton and Tharp (43) to be produced from milk fat during steam distillation. Parks et al. (39) isolated the methyl ketone precursor from butter fat by passing 100.6 g of butter oil being made up to 500 ml with hexane through a chromatographic column containing 30 g of a 2:1 mixture of Celite 545 (Johns-Manville Company, New York) and Magnesia 2665 (Fisher Scientific Company, Silver Spring, Md.).

The mechanisms for the formation of methyl ketones in milk fat are not completely understood. There is, however, sufficient evidence to show that the methyl ketones of odd-numbered carbons from C_3 to C_{15} are a result of a nonoxidative mechanism and are not due to an autoxidation breakdown (18).

B. Separation techniques.

There has been a gradual change in the approach to separation and identification of the components of aroma over the past twenty-five years, from column and thin layer

chromatography coupled with chemical and physical methods of identification, to gas chromatography (GLC) for separation and the mass spectrometry for identification.

GLC has been used successfully for separating chemical compounds associated with flavors in foods for more than 20 years. McGugan and Howsam (38), however, emphasized the following concern that the analyst should be aware of: During chromatography of food flavor volatiles, essential components of the flavor may be absorbed or decomposed by GLC column, hence the total effluent should be trapped and compared for aroma with the unchromatographed sample. They found for example that when cheddar cheese volatiles were chromatographed on several different columns, the condensible material recovered from the total effluent did not smell like the sample of volatiles before it was chromatographed. The columns exhibiting this phenomenon, Carbowax 20M on Chromosorb W-HP and uncoated Porapak Q are frequently used in the analysis of flavor volatiles. The aromas of cheddar volatiles recovered from two other columns indistinguishable from the aromas of unchromatographed volatiles were stainless, 183 cm x 2 mm i.d., packed with 10% OV 225 on 80/100 mesh Chromosorb W-HP and 183 cm x 5 mm i.d., packed with 10% OV 101 + 0.5% Igepal CO 880 on 80/100 mesh Chromosorb W-HP. For measuring volatile compounds in biological fluids, including milk, Bassette et al. (6, 7, 8, 9, 10, 11) successfully used 304.8 x 0.318 cm stainless steel columns packed with 20% Carbowax 20M on 60/80 mesh, acid washed HMDS-treated

Chromosorb P to analyze the following: 1). A solution containing 18 components of a complex mixture of sulfides, carbonyls, esters, and alcohols at the 1 ppm level. 2). Some volatile materials in biological solutions (e.g., water and milk). 3). Acetone content in human blood and urine related to fasting, diabetes, and tests of blood glucose tolerance. 4). Parts per billion levels of carbonyls added to milk. 5). Some volatile compounds (acetal, methyl sulfide, propanal, n-pentanal, and n-hexanal) in the level of parts per billion from milk before and after light or copper exposure.

C. Identification.

Gas chromatography has been used primarily for the separation of chemical compounds. However, GC retention times also may be used to identify the separated compounds by comparing them with reference compounds under several conditions of isothermal or programmed-temperature gas chromatography. When measuring volatile compounds from autoxidized milk lipids, the reference compounds (alkanals, 2-alkenals, 2,4-alkadienals, etc.) are recommended (2). Bassette et al. (8) demonstrated that in addition to retention times, evidence for identification of the trace organic compounds (sulfides, carbonyls, esters, and alcohols) was obtained by eliminating peaks by subtractive techniques with selective qualitative reagents. In a review, Evans (21) reported that mass spectrometry (MS) has most to offer in the identification of compounds separated by GLC. Any sample which

can be separated by GLC can be expected to be run satisfactorily in a MS. The MS must be operated at very low pressure (10^{-7} torr) while the effluent from the GLC is at atmospheric pressure. Introducing a component from the GLC gas stream into the MS at the low pressure requires an intermediate enrichment of the organic material in the gas stream by selectively removing the GLC carrier gas. This is being done successfully with several types of molecular separators in which the enriched gas stream then is fed into the ionization chamber of the MS under reduced pressure. Perhaps the greatest deterrent to the use of MS-GLC combinations in flavor evaluation is the high cost of this instrumentation. Although it is being used in research laboratories, few quality control labs could afford it in routine quality assurance work. Evans also described combining GLC/infrared (IR) or GLC/nuclear magnetic resonance (NMR) to identify compounds in the GLC eluant as less effective alternatives.

IV. Previous Work in Studying Induced Off-Flavors by Exposing Component Parts of Milk to the Flavor Provoking Agents.

Singleton et al. (50) dialyzed skim milk (1 vol) against distilled water (2 vol) through cellophane membrane for 48 h then, the component parts (dialyzable and nondialyzable components) of skim milk were exposed to direct sunlight at midday. After organoleptic examinations of flavor intensity of light exposed milk components, they concluded that the flavor

was nondialyzable. Gawel and Pijanowski (24) demonstrated that oxidative changes of phospholipids of skim milk after adding 1.5 ppm Cu and reacting for 3 days resulted in off-flavors. They also found that only 1.4 mg O_2/g of total extracted lipids, compared with up to 200 mg O_2/g of extracted phospholipids. Forss, Pont and Stark (22) mentioned that the light-induced flavor is caused chiefly by oxidation products from the phospholipids in milk, skim milk and whey. Storgard and Lindqvist (51) separated skim milk into casein and serum (whey) by centrifugation at 40,000 rpm for one hour at 15 C. The casein and serum with or without light exposure then were recombined crosswise to simulate milk and determine which fraction contained the light-induced off-flavor. They concluded that serum protein, although probably not the only factor, was a more significant source of the substances causing light-induced flavor than the milk's casein. However, there is no references in the literature concerning the changes of volatile constituents in milk or fractions of milk exposed to light or added copper except the two papers by Bassette (6, 7).

EXPERIMENTAL

Chapter I. Apparatus, Materials, and Methods

Procedure.

Steam distillation.

Steam distillation in digestion-distillation flasks of an improved Kemmerer-Hallett type micro-Kjeldahl nitrogen distillation apparatus was used to isolate volatile materials from milk. This apparatus was first pre-cleaned by refluxing 5 min with boiled distilled water. Fifty milliliters of milk or milk fractions at or below 10 C and one drop of GE antifoam 66 (100% active silicone defoamer) then were transferred to the dry distillation flask. An 11-cm, tapered glass tube was connected to the outlet tube of the condenser so the tip fit within 3 mm of the bottom of a 15-ml conical test tube. One-half milliliter of distilled water was added to the conical collection test tube, which was positioned in an ice-water bath with the condenser outlet extension tube below the water's surface. The bath ice-water was maintained well above a 5-ml graduation mark on the test tube. Exactly 5 ml $\pm$ 0.1 ml of distillate was collected within 5 to 6 min. Two 2-ml aliquots of distillate from the tube were pipetted rapidly into 5-ml serum vials, which contained 1.2 g of crystalline sodium sulfate. These samples were stored at 4 C until they were analyzed by GLC. In preparation for GLC analysis the serum vial was sealed with a rubber serum cap and placed into a 60 C water bath for 2 min. The charged vial then was transferred to a mechanical horizontal-

general purpose shaker (Eberbach & Sons) and shaken for 5 min. The serum cap of the vial was changed quickly to avoid getting liquid on the needle when sampling and the vial was returned to the 60 C water bath for 8 min. Finally, 1 ml of vapor was removed from the vial with the 1-ml Hamilton #1001 gas tight syringe with a 25 gauge needle and injected into the GLC instrument.

GLC analysis.

In this research Varian Aerograph model 500-C fitted with a hydrogen flame detector was used with a 1-mv. Brown-Honeywell recorder. A stainless steel column (3.5m X 3.18mm) packed with 20% Carbowax 20 M on 60/80 mesh acid-washed HMDS-treated Chromosorb P was employed in the instrument. Operating conditions were: column temperature, 100 C; gas flow (ml/min): nitrogen, 17.2, hydrogen, 26.0, oxygen, approximately 80. Sample bottles for the distillate were serum vials, 15mm in diameter X 52mm, of 5-ml capacity with self-sealing rubber caps.

Standard curves.

According to Bassette and Ward (10) GLC responses were quantified by preparing and analyzing milk samples containing 0, 5, 10, 25, and 50 ppb each of added acetal, methyl sulfide, propanal, n-pentanal, and n-hexanal. After subtracting peak heights from the analyses of milk or milk fraction with nothing added, peak heights were calculated for each of the spiked samples. Each GLC peak height was from the average of two injections.

Results.

Fig. 1-5 illustrate the linear relationships between peak heights from the complete analyses of the milk samples to which from 5 to 50 ppb of certain volatile materials had been added. The regression equations for the lines are in Table 1.

Table 1. Regression equations for volatile materials added to milk in parts per billion (ppb)

Compounds	Regression equations[a,b,c]
Acetal	$Y = 0.37\ X + 0.30$
Methyl sulfide	$Y = 0.14\ X + 1.85$
Propanal	$Y = 0.10\ X + 1.38$
n-Pentanal	$Y = 1.25\ X + 1.23$
n-Hexanal	$Y = 0.67\ X + 0.48$

[a] Y = concentration in ppb.

[b] X = % of recorded full scale deflection X attenuation factor in GLC analysis.

[c] Instrument sensitivity was adjusted for daily variations by analyzing a standard 1-ppm acetone solution and adjusting the response so that it yielded a peak height of 800 (recorder deflection X attenuation).

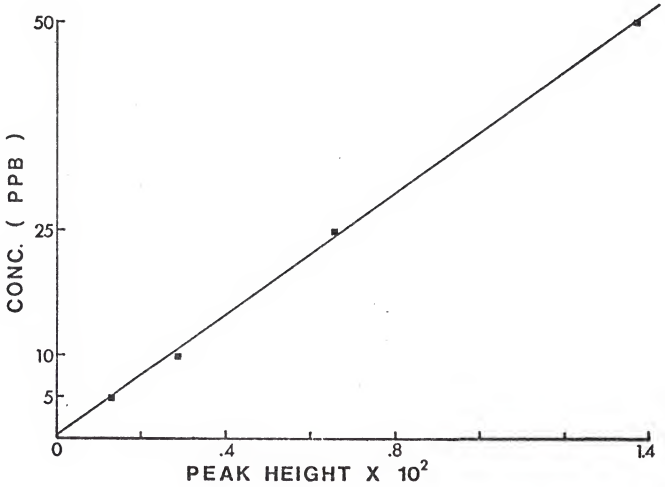

Fig. 1. Standard curve of acetal in milk from steam distillation GLC analyses.

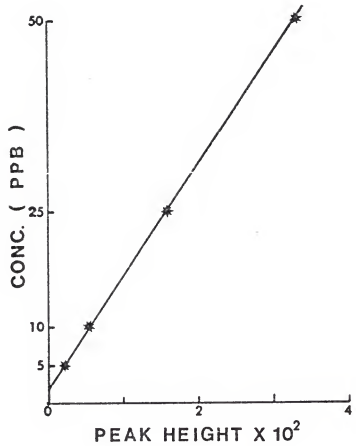

Fig. 2. Standard curve of methyl sulfide in milk from steam distillation GLC analyses.

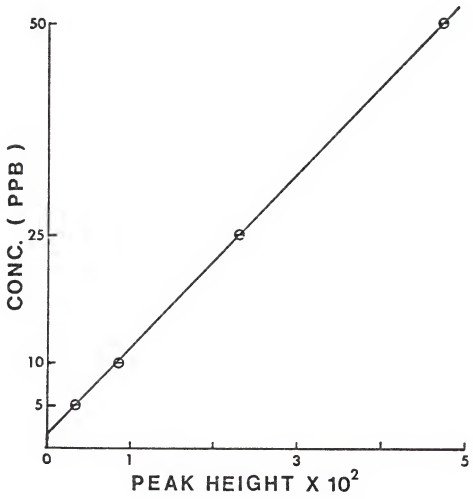

Fig. 3. Standard curve of propanal in milk from steam distillation GLC analyses.

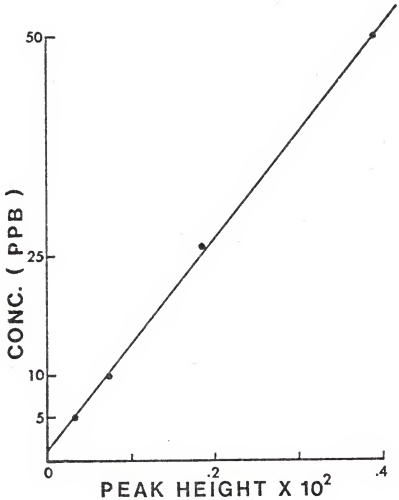

Fig. 4. Standard curve of n-pentanal in milk from steam distillation GLC analyses.

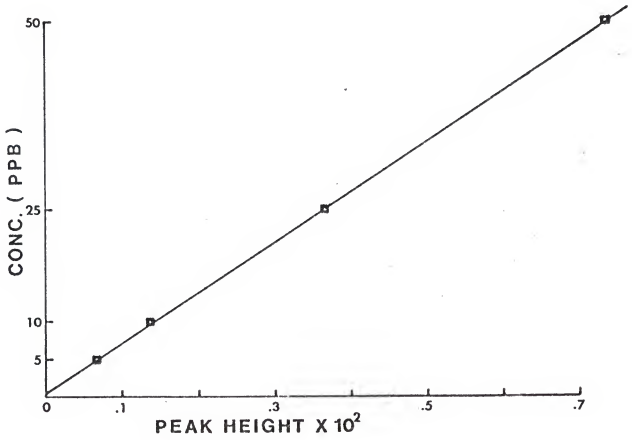

Fig. 5. Standard curve of n-hexanal in milk from steam distillation GLC analyses.

Chapter II. Effects of Light on Concentrations of Some Volatile Materials in Milk.

Procedure.

About 400 ml of raw milk was collected from the surge tank of a De Laval Vacu-Therm pasteurizer at the University Dairy Plant during routine milk processing. Another sample of about 200 ml from the same raw milk supply was obtained from a carton about 1-hr after it was plant-pasteurized (76.6 C/15 sec) with Vacu-Therm treatment and homogenized in a CP homogenizer under 1500 psi using a single-service, conical homo valve. About half of the raw milk was transferred to a 500-ml Erlenmeyer flask and laboratory pasteurized at 63 C for 30 min with continuous gentle agitation. Each half was divided again into two equal parts: a control and a sample exposed at 5 C in a clear-glass Erlenmeyer flask to midday sunshine for 30 min. Distillations for GLC analyses were made within 4 h after light exposure. GLC analyses were made on the day milks were distilled.

Results and Discussion.

Susceptibilities of raw, laboratory pasteurized, and plant-pasteurized homogenized milks from the same milk supply to light activation were compared in Table 2. Concentrations of each chemical component in all control milks were similar; n-pentanal varied most. Of all the volatile materials, acetal, propanal, n-pentanal, and n-hexanal were studied because of their relation to light- and copper-induced changes. Of the four carbonyl compounds analyzed, n-pentanal changed more with light

exposure than any of the others. This was especially true for the homogenized-pasteurized milk. The concentration ratios of n-pentanal in raw, laboratory pasteurized, and plant-pasteurized homogenized milks of with/without light exposure were 5.7, 4.1 and 7.2, respectively. Others (6, 54d) also have reported that homogenized milk is more susceptible to light-induced changes than non-homogenized milk.

TBA values according to the method of King (35), a measure of lipid oxidation, changed most in the plant-pasteurized homogenized milk (0.033 to 0.051). Greater sensitivity to light-induced changes may be due to larger total surface area of fat globules after homogenization and consequently, the total photoenergy received is greater than that by non-homogenized milks.

According to Table 2, none of the TBA values was greater than 0.055, which Aurand et al. (1) suggested would be the minimum value for lipid oxidation in milk after 20 min sunlight exposure.

Table 2. Concentrations of some volatile aldehydes from raw, laboratory pasteurized, and plant-pasteurized homogenized milks with and without sunlight exposure.

Treatment	Raw milk	Lab. past. milk[a]	Plant past. milk[b]
	Acetal[c] (ppb)		
0	9.5	9.5	9.5
30 min	42.5	46.1	49.8
	Propanal[c] (ppb)		
0	2.0	2.4	2.0
30 min	5.5	6.1	6.6
	n-Pentanal[c] (ppb)		
0	20.0	32.2	26.0
30 min	113.6	126.1	188.5
	n-Hexanal[c] (ppb)		
0	14.7	20.3	17.0
30 min	61.0	68.4	54.2
	TBA values		
0	0.033	0.035	0.033
30 min	0.041	0.045	0.051

[a]63 C for 30 min.

[b]Vacu-Therm, HTST pasteurized, homogenized.

[c]Values are from the average of duplicate analyses.

Chapter III. Effects of Copper on Concentrations of Some Volatile Materials in Milk

Procedure.

Raw, laboratory pasteurized, and plant-pasteurized homogenized milk were collected as described in Chapter II. Cupric sulfate was added to aliquots of each of the three milks to give a concentration of 5 ppm copper. A portion of each milk was stored in a refrigerator at 4 C for 3 days, then analyzed for volatile materials. The 2-thiobarbituric acid (TBA) test was run on the samples by the method of King (35). Distillations for GLC analyses were made within 4 h after three reaction days. GLC analyses were run on the same day.

Results and Discussion.

Table 3 shows the concentrations of some volatile materials in raw, laboratory pasteurized, and plant-pasteurized homogenized milks exposed to 5 ppm copper for 3 days. The concentrations of each of the chemical compounds in the control milk did not differ appreciably with different processing conditions. n-Hexanal increased more than any other volatile compound measured in milk exposed to copper. Concentrations of the four carbonyl compounds monitored were lower in the copper-treated plant-pasteurized homogenized sample; perhaps because the homogenization during Vacu-Therm treatment of the milk, reduced the dissolved oxygen as suggested by Bassette (7).

TBA values of raw, laboratory pasteurized, and plant-pasteurized homogenized milks exposed to copper were 0.156,

0.207 and 0.131, respectively. The relatively lower TBA value for the plant-pasteurized homogenized sample than for the other samples exposed to copper generally paralleled the lower concentration of volatile materials.

Table 3. Concentrations of some volatile aldehydes from raw, laboratory pasteurized, and plant-pasteurized homogenized milks with and without added copper.

Treatment	Raw milk	Lab. past. milk[a]	Plant past. milk[b]
		Acetal[c] (ppb)	
Control	2.2	4.0	4.0
Cu (5 ppm)	37.0	40.6	31.4
		Propanal[c] (ppb)	
Control	---	---	---
Cu (5 ppm)	66.4	90.5	57.7
		n-Pentanal[c] (ppb)	
Control	13.6	26.0	19.8
Cu (5 ppm)	581.9	619.3	563.1
		n-Hexanal[c] (ppb)	
Control	---	---	---
Cu (5 ppm)	1873.7	1913.8	1606.1
		TBA values	
Control	0.029	0.031	0.031
Cu (5 ppm)	0.156	0.207	0.131

[a]63 C for 30 min.

[b]Vacu-Therm, HTST pasteurized, homogenized.

[c]Values are from the duplicate analyses.

Chapter IV. Effects of Sodium Sulfite and Various Combinations of Antioxidants in Inhibiting Oxidation of Milk Exposed to Light

Procedure.

To test the effect of sodium sulfite (10^{-3}M) as an antioxidant for milk exposed to light, three 100 ml aliquots of plant pasteurized milk were treated as follows: one with added Na_2SO_3 and no light exposure (the control), one with added Na_2SO_3 and light exposure, the third one was subjected to fluorescent light without added Na_2SO_3. Each sample was put in a 250-ml glass Erlenmeyer flask. Except the control milk, the samples were exposed to fluorescent light placed at 4 C for 12 h in a commercial milk display case. The milk was placed 30.5 cm from and perpendicular to the light source at the midpoint of the exposed horizontal surface.

The plant-pasteurized homogenized milk served as a control for evaluating various combinations of antioxidants. The antioxidants employed included butylated hydroxyanisole (BHA), ethylenediamine tetraacetic acid (EDTA), sodium citrate, butylated hydroxytoluene (BHT) and propyl gallate (PG), in concentrations of .01% (w/v). The following seven samples were studied: 1. Control milk. 2. Milk + BHA + EDTA. 3. Milk + BHA + PG + sodium citrate. 4. Milk + BHA + BHT. 5. Milk + BHA + PG. 6. Milk + BHA + BHT + sodium citrate. 7. Milk. All the milk samples except the control were exposed to sunlight for 30 min during midday.

The distillations of the milk and GLC analyses were the same as previously described. Changes in concentration of

some volatile carbonyl compounds (i.e., acetal, propanal, n-pentanal and n-hexanal) in parts per billion were calculated from their regression equations.

Results and Discussion.

Na_2SO_3 reduced the effect of light. Fig. 6 shows that Na_2SO_3 was effective in reducing concentrations of acetal, propanal, and especially n-pentanal, but not n-hexanal. Increases in concentrations of C_2, C_3, C_5 & C_6 aldehydes in milk treated with Na_2SO_3 and exposed to fluorescent light over those in the control were 11.0, 0.5, 68.6 and 30.4 ppb. The same treatment less the Na_2SO_3 resulted in increases in concentrations of 14.6, 1.5, 162.4 and 30.4 ppb over the control. Since Na_2SO_3 was not effective in reducing the concentration of n-hexanal after light exposure, its production must be by a mechanism different from that involving n-pentanal.

Of the combinations of antioxidants studied, BHA + PG + sodium citrate was most effective in inhibiting light-induced oxidation (see Fig. 7). The concentrations of C_2, C_3, C_5 & C_6 aldehydes in milk with the addition of BHA + PG + sodium citrate and exposed to light were 5.0, 0.8, 37.5 and 3.3 ppb greater than those of control milk (without light and antioxidant). Milk without these antioxidants and exposed to light contained 16.5, 4.6, 218.6 and 80.2 ppb more of the carbonyl compounds than did the control.

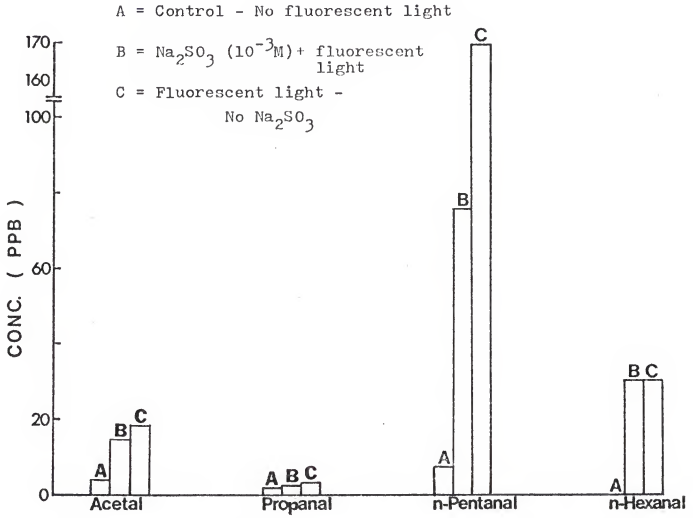

Fig. 6. Concentrations of Acetal, Propanal, n-Pentanal, and n-Hexanal in milk treated with Na_2SO_3 and exposed to fluorescent light.

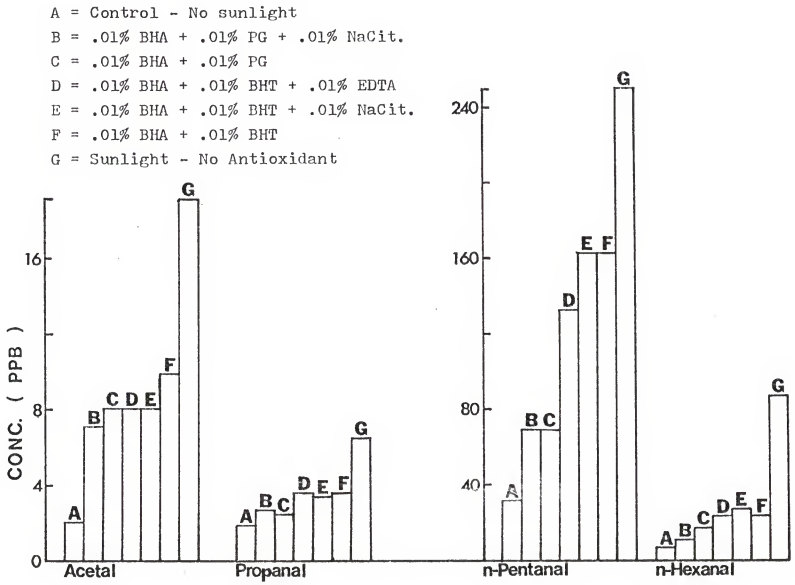

Fig. 7. Concentrations of Acetal, Propanal, n-Pentanal, and n-Hexanal in milk treated with various antioxidants and exposed to sunlight.

Chapter V. Effects of Sodium Sulfite and Various Combinations of Antioxidants in Inhibiting Oxidation of Milk Exposed to 5 ppm Copper

Procedure.

Three samples of 100 ml each of plant pasteurized milk were placed in 250 ml glass Erlenmeyer flasks. One sample with nothing added served as a control, the second sample contained 10^{-3}M Na_2SO_3 and 5 ppm copper, and the third contained 5 ppm copper. The three milk samples were stored at 4 C in dark for 3 days.

The same six combinations of antioxidants and control milk used to study light-induced oxidation (Chapter IV), were employed to study copper-induced oxidation.

Results and Discussion.

Although Na_2SO_3 had an inhibitory effect against copper induced oxidation, it would be of little value from a practical point of view, since a considerable amount of oxidation still remained and Na_2SO_3 contributed an objectionable flavor. Surprisingly n-propanal was inhibited more than the other carbonyl compounds. The concentration of C_2, C_3, C_5 & C_6 aldehydes in milk with added Na_2SO_3 and 5 ppm copper exposure increased 18.2, 3.5, 93.1 and 114.2 ppb while those in milk without added Na_2SO_3 but with copper exposure increased by 42.0, 63.4, 455.8 and 1047.5 ppb (see Fig. 8).

Of the antioxidant combinations studied, BHA + EDTA was most effective in inhibiting oxidation in milk exposed to copper

(see Fig. 9). Increases in concentrations of acetal, propanal, n-pentanal, and n-hexanal from the milk samples with copper, BHA, and EDTA added were 1.8, 1.9, 12.4 and 3.4 ppb, whereas those without the BHA and EDTA were 40.2, 57.7, 624.3 and 1348.1 ppb. EDTA is a strong metal chelating agent which would be expected to chelate most of the added copper and consequently inhibit copper-induced oxidation.

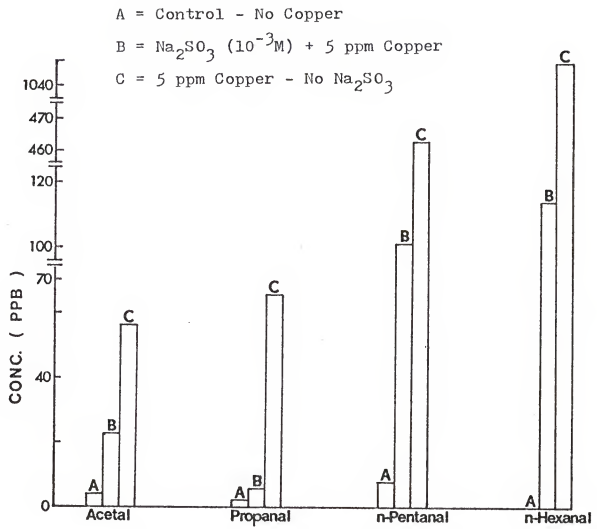

Fig. 8. Concentrations of Acetal, Propanal, n-Pentanal, and n-Hexanal in milk treated with Na_2SO_3 and exposed to copper.

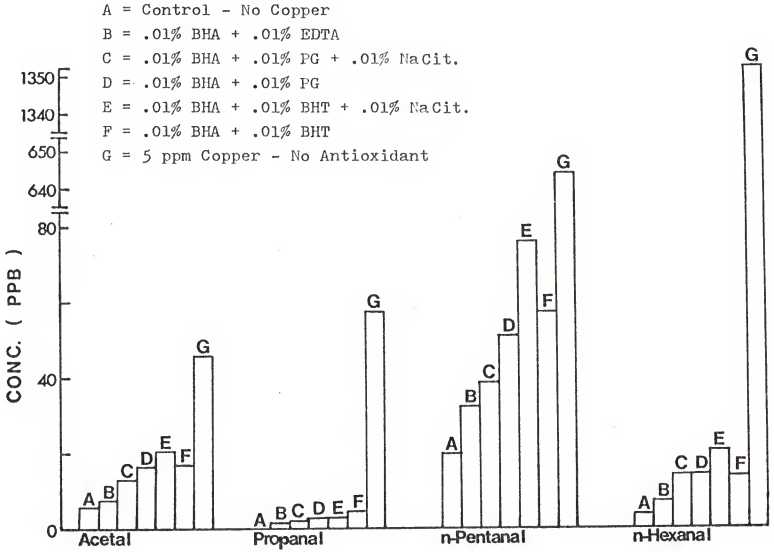

Fig. 9. Concentrations of Acetal, Propanal, n-Pentanal and n-Hexanal in milk with various antioxidants and exposed to copper.

Chapter VI. Susceptibility of Fractions of Laboratory Pasteurized Milk to Light-Induced and Copper-Induced Changes

Procedure.

Laboratory pasteurized milk for this experiment was prepared by heating raw milk in an Erlenmeyer flask to 63 C for 30 min with agitation. It then was cooled to 4 C and centrifuged at 2000 rpm for 30 min to separate cream from skim milk. The skim milk fraction was acidified with 1.0 N HCl to pH 4.6 and casein separated from the acid whey. The whey and precipitated casein were neutralized to pH 6.7 with 1.0 N NaOH. The cream, skim milk, neutralized whey and casein then were resuspended in boiled distilled water to the original volume of laboratory pasteurized milk.

Equal volumes of neutralized whey, skim milk, resuspended cream, and resuspended casein based on the original milk volume together with laboratory pasteurized milk were exposed to fluorescent (F40CW) light for 51 h. An identical series of samples were treated with 5 ppm copper but reacted for 3 days in the dark. The ratios of concentrations of acetal, propanal, n-pentanal, and n-hexanal of with/without light or copper exposure from each fraction of laboratory pasteurized milk were determined after steam distillations and GLC analyses.

Results and Discussion.

Fig. 10 shows that neutralized whey was more susceptible to light-induced changes than any other fraction. The ratios

of concentrations of the C_2, C_3, C_5 & C_6 aldehydes (4.8, 6.5, 6.2 and 47.3) of with/without light exposure from neutralized whey were greatest. For the copper-induced oxidation, resuspended cream was most susceptible and neutralized whey next (see Fig. 11). The concentration ratios of those aldehydes from resuspended cream of with/without copper exposure were 14.6, 33.5, 28.6 and 86.0 (in the order of C_2, C_3, C_5 and C_6 aldehydes).

Since neutralized whey contains only about 0.03% ether extractable lipid material, its susceptibility to oxidative deterioration may have been due to highly unsaturated fatty acids of the lipid or phospholipid components. Conceiveably, the aldehydes may have come from other precursors.

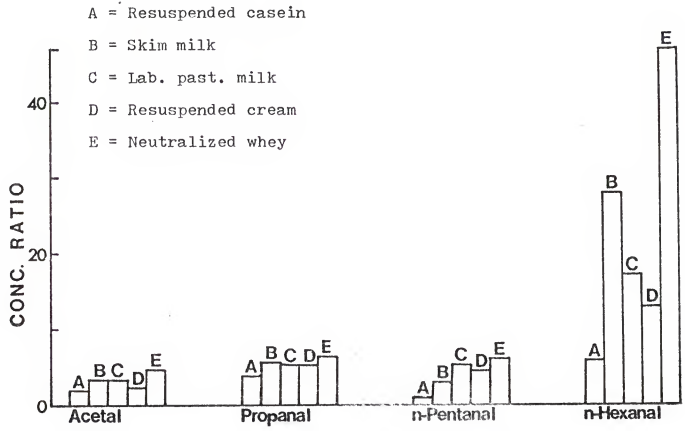

Fig. 10. Concentration ratio of Acetal, Propanal, n-Pentanal, and n-Hexanal of with/without fluorescent light exposure in laboratory pasteurized milk fractions.

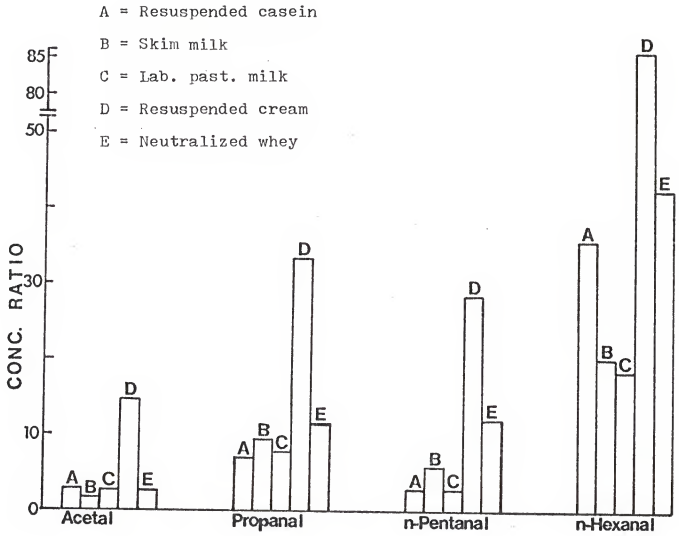

Fig. 11. Concentration ratio of Acetal, Propanal, n-Pentanal, and n-Hexanal of with/without copper exposure in laboratory pasteurized milk fractions.

Chapter VII. Susceptibility of Component Parts of Neutralized Whey from Laboratory Pasteurized Milk to Light-Induced and Copper-Induced Changes

Procedure.

Fifty milliliter aliquots of neutralized whey (pH 6.7) were dialyzed through cellophane membranes at 4 C in dark for 48 h individually against 150 ml boiled distilled water, with continuous magnetic stirring.

Equal volumes of neutralized whey, dialyzed whey, and the dialysate were exposed to cool white, 40 watt fluorescent light for 24 h. The light source was 25.5 cm perpendicular to the milk surface at the mid-point of the exposed horizontal surface. An identical series of samples was exposed to 5 ppm copper at 4 C for 3 days. Fifty milliliters of each fraction was subjected to distillation and subsequent analysis by GLC. The resulting calculated-concentrations of the carbonyl compounds of dialysate were multiplied by 4 because of the dilution attained with dialysis (150 ml of boiled distilled water against 50 ml neutralized whey during the dialysis).

Results and Discussion.

Except for acetal, the dialyzed whey fraction was most susceptible to light-induced changes. This is shown by ratios of concentrations of with/without light: those ratios of C_2, C_3, C_5 and C_6 aldehydes were 3.5, 2.4, 2.0 and 1.7, respectively from dialyzed whey, compared to 7.7, 1.3, 0.9 and 1.1, respectively from the dialysate (see Fig. 12). Note: a value of

1.0 indicates no effect from exposure to light.

The dialyzed whey was most susceptible to copper-induced changes (see Fig. 13). The ratios of concentrations of C_2, C_3, C_5 and C_6 aldehydes of with/without copper exposure were 8.9, 17.3, 12.9 and 16.5, respectively from dialyzed whey compared to 5.6, 1.2, 1.1 and 1.0, respectively from the dialysate.

The concentration ratios of those aldehydes from neutralized whey of laboratory pasteurized milk origin were lower than those from dialyzed whey from the same milk in both light- and copper-induced changes experiments.

Except for acetal in the case of light-induced changes (see Fig. 12), the precursors of the aldehydes are mainly non-dialysable.

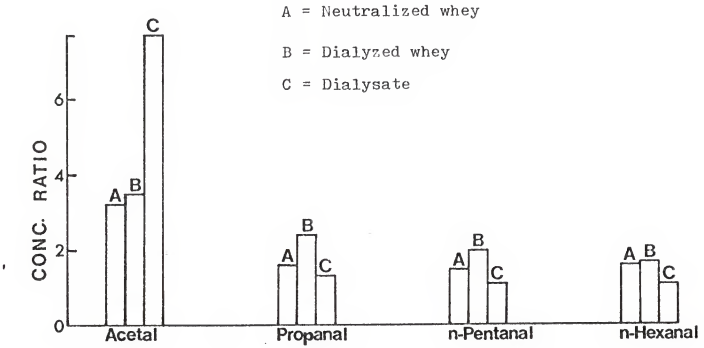

Fig. 12. Concentration ratio of Acetal, Propanal, n-Pentanal, and n-Hexanal of with/without fluorescent light exposure in neutralized whey fractions from laboratory pasteurized skim milk.

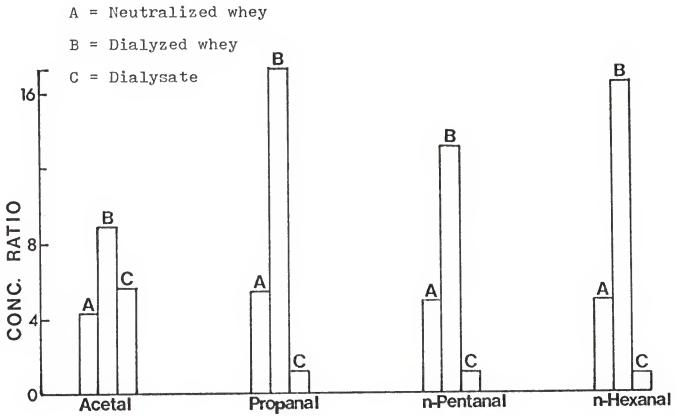

Fig. 13. Concentration ratio of Acetal, Propanal, n-Pentanal, and n-Hexanal of with/without copper exposure exposure in neutralized whey fractions from laboratory pasteurized skim milk.

Chapter VIII. Susceptibility of Neutralized Whey from a Retail Commercial Skim Milk and its Dialyzed Fractions to Light-Induced and Copper-Induced Changes

Procedure.

The procedures for obtaining neutralized whey, dialyzed whey, and dialysate were the same as those for the laboratory pasteurized milk described in the previous experiment. However, in addition, two 50-ml aliquots of the dialysate were distilled separately and 5-ml distillates collected from each were diluted to the original 50 ml. One of the diluted distillates served as a control without light or copper exposure. The pot solution was the material that remained in the micro-Kjeldahl flask after the distillation of dialysate.

A series of samples of skim milk, neutralized whey, dialyzed whey, dialysate, diluted distillate, and the distillation-pot solution obtained from a retail commercial skim milk was subjected to cool white, 40 watt fluorescent light or 5 ppm copper exposure at 4 C for 24 h and 3 days, respectively. Another set of those fractions without light or copper exposure served as control.

Following steam distillations and GLC analyses of the C_2, C_3, C_5 and C_6 aldehydes, the concentration ratios of those carbonyl compounds of with/without light or copper exposure from each fraction were determined.

Results and Discussion.

Fig. 14 shows results from light-induced changes of the commercial skim milk. The concentration ratios of acetal and

propanal from dialyzed whey (3.2, 1.9) were only slightly less than those from neutralized whey (4.1, 2.4); while those of n-pentanal and n-hexanal from dialyzed whey (2.9, 3.0) were slightly greater than those from neutralized whey (2.0, 1.6). The concentration ratios of C_2, C_3, C_5 and C_6 aldehydes from both dialyzed and neutralized whey were greater than those from dialysate and diluted distillate. Surprisingly, the ratios of acetal and n-pentanal from distillation-pot solution were quite high as compared to the other fractions of the commercial skim milk: 9.7 for acetal and 8.5 for n-pentanal. With regard to this phenomenon, some precursors of C_2 and C_5 aldehydes were dialysable but not distillable.

In regard to the copper-induced changes of the commercial skim milk, the concentration ratio of n-hexanal from dialyzed whey (15.1) was less than that from neutralized whey (26.3); while that of acetal from dialyzed whey (4.7) was slightly greater than that from neutralized whey (4.0). Furthermore, the concentration ratios of propanal and n-pentanal from dialyzed whey (4.2, 7.8) were almost the same as those from neutralized whey (4.4, 7.7). It was the whey fractions of the commercial skim milk that contribute most to copper-induced oxidation (see B & C in Fig. 15). However, the concentrations of C_2, C_3, C_5 and C_6 aldehydes increased in distillation-pot solution after copper exposure by 23.7, 6.0, 37.4 and 20.5 ppb, respectively over the control. The concentration ratios of acetal and n-pentanal of with/without copper exposure from distillation-

pot solution were 4.1 and 3.3, also indicating some precursors of the four carbonyl compounds were dialysable but not distillable.

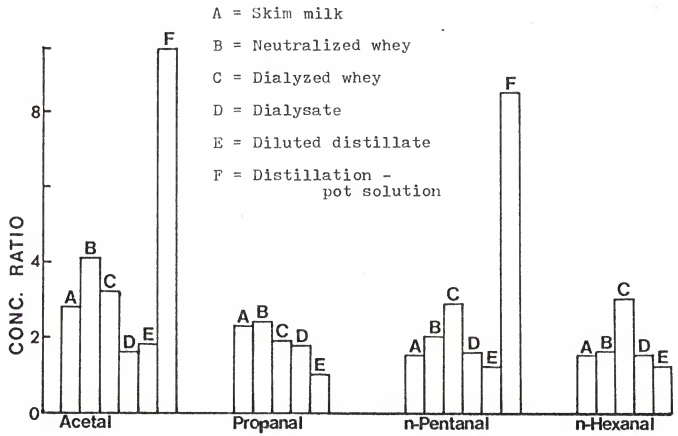

Fig. 14. Concentration ratio of Acetal, Propanal, n-Pentanal, and n-Hexanal of with/without fluorescent light exposure in neutralized whey fractions from a commercial pasteurized homogenized skim milk.

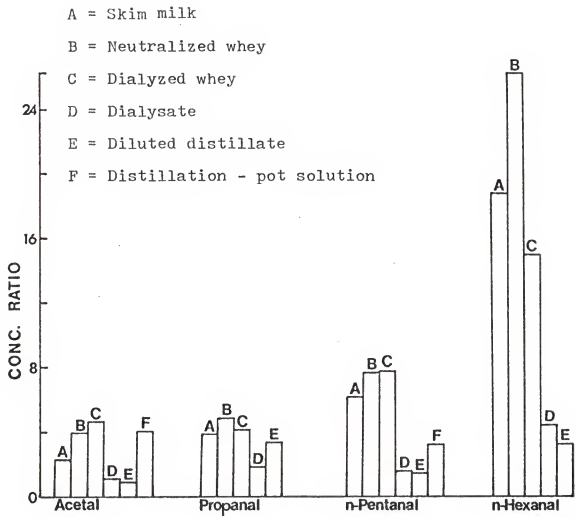

Fig. 15. Concentration ratio of Acetal, Propanal, n-Pentanal, and n-Hexanal of with/without copper exposure in neutralized whey fractions from a commercial pasteurized homogenized skim milk.

CONCLUSIONS

Based on the data presented, the following conclusions may be drawn:

1. The TBA test was ineffective in measuring light induced changes in milk.
2. Na_2SO_3 (10^{-3}M) was not an effective antioxidant for milk. Furthermore, it contributed a strong sulfur flavor to the milk.
3. The combination of BHA, PG, and sodium citrate were most effective among the various combinations of antioxidants studied in inhibiting light-induced changes while BHA and EDTA were most effective in inhibiting copper-induced changes as measured by GLC.
4. Neutralized whey (pH 6.7) from a laboratory pasteurized milk were more susceptible to light-induced changes than skim milk, laboratory pasteurized milk, resuspended cream, and resuspended casein. Resuspended cream was more susceptible to copper-induced changes than any other fraction.
5. Except for acetal, dialyzed whey and not the dialysate from laboratory pasteurized milk contributed most to light-induced and copper-induced changes. The dialyzed whey from a retail commercial skim milk contributed more to light-induced and copper-induced changes than those from dialysate of the same batch of commercial skim milk. The precursors of carbonyl compounds remained in distillation-pot solution for copper-induced changes.

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APPENDIX A

Table 4. Concentrations of acetal, propanal, n-pentanal, and n-hexanal in milk exposed to fluorescent light and treated with 10^{-3}M Na_2SO_3

Control milk[a]	Exposed to fluorescent light	
	Milk[a] + Na_2SO_3	Milk[a]
	Acetal[b] (ppb)	
4.0	15.0	18.6
	Propanal[b] (ppb)	
2.0	2.5	3.5
	n-Pentanal[b] (ppb)	
7.4	76.2	169.8
	n-Hexanal[b] (ppb)	
---	30.4	30.4

[a]Without Na_2SO_3

[b]Concentrations represent average of duplicate GLC analyses for each distillation.

Table 5. Concentrations of acetal, propanal, n-pentanal, and n-hexanal in milk exposed to 5 ppm Cu and treated with 10^{-3}M Na_2SO_3.

Control milk[a]	5 ppm Cu in milk	
	Milk + Na_2SO_3	Milk
	Acetal[b] (ppb)	
4.0	22.2	46.0
	Propanal[b] (ppb)	
2.0	5.5	65.4
	n-Pentanal[b] (ppb)	
7.4	101.1	463.2
	n-Hexanal[b] (ppb)	
---	114.2	1047.5

[a]Without Na_2SO_3

[b]Concentrations represent average of duplicate GLC analyses for each distillation.

Table 6. Concentrations of acetal, propanal, n-pentanal, and n-hexanal in milk with various combinations of antioxidants and exposed to sunlight.

	Exposed to sunlight					
Control milk[a]	Milk[a] + BHA PG Na-citrate	Milk[a] + BHA PG	Milk[a] + BHA EDTA	Milk[a] + BHA BHT Na-citrate	Milk[a] + BHA BHT	Milk[a]
		Acetal[b] (ppb)				
2.1	7.1	8.1	8.1	8.1	8.9	18.6
		Propanal[b] (ppb)				
1.9	2.7	2.5	3.6	3.4	3.6	6.5
		n-Pentanal[b] (ppb)				
32.4	69.9	69.9	132.4	163.6	163.6	251.0
		n-Hexanal[b] (ppb)				
7.2	10.5	17.2	23.9	27.2	23.9	87.4

[a]Without antioxidant.

[b]Concentrations represent average of duplicate GLC analyses for each distillation.

Table 7. Concentrations of acetal, propanal, n-pentanal, and n-hexanal in milk with various combinations of antioxidants and exposed to 5 ppm Cu.

	5 ppm Cu in milk					
Control milk[a]	Milk + BHA EDTA	Milk + BHA PG Na-citrate	Milk + BHA PG	Milk + BHA BHT Na-citrate	Milk + BHA BHT	Milk
		Acetal[b] (ppb)				
5.8	7.6	13.1	16.7	20.4	16.7	46.0
		Propanal[b] (ppb)				
---	1.9	2.4	2.9	2.9	4.4	57.7
		n-Pentanal[b] (ppb)				
20.0	32.4	38.7	51.2	76.2	57.4	644.3
		n-Hexanal[b] (ppb)				
3.8	7.2	13.9	13.9	20.5	13.9	1351.9

[a]Without antioxidant.

[b]Concentrations represent average of duplicate GLC analyses for each distillation.

Table 8. Concentrations of acetal, propanal, n-pentanal, and n-hexanal in laboratory pasteurized milk fractions with and without fluorescent light exposure.

Treatments	Milk fractions				
	Resuspended[d] casein	Skim milk	Lab. past.[c] milk	Resuspended[d] cream	Neutralized[e] whey
		Acetal[f] (ppb)			
0[a]	14.9	14.9	14.9	7.6	14.9
51h[b]	27.7	53.3	53.3	18.6	71.5
		Propanal[f] (ppb)			
0	2.9	3.9	3.4	2.9	3.9
51h	11.6	21.9	18.3	15.7	25.4
		n-Pentanal[f] (ppb)			
0	82.4	44.9	38.7	38.7	38.7
51h	86.7	138.6	213.5	182.3	238.5
		n-Hexanal[f] (ppb)			
0	23.9	17.2	20.5	17.2	20.5
51h	141.0	482.2	351.7	221.2	970.5

[a]Without light exposure.

[b]With F40CW light exposure at 4 C for 51 h.

[c]63 C,30 min.

[d]Resuspended in boiled distilled water to the original volume of laboratory pasteurized milk.

[e]pH 6.7.

[f]Concentrations represent average of duplicate GLC analyses for each distillation.

Table 9. Concentrations of acetal, propanal, n-pentanal, and n-hexanal in laboratory pasteurized milk fractions with and without copper exposure.

Treatments	Milk fractions				
	Resuspended[d] casein	Skim milk	Lab. past.[c] milk	Resuspended[d] cream	Neutralized[e] whey
		Acetal[f] (ppb)			
Control[a]	11.2	29.5	25.9	3.9	25.9
5 ppm Cu[b]	31.3	49.6	69.7	56.9	69.7
		Propanal[f] (ppb)			
Control	2.9	5.5	7.5	2.9	5.0
5 ppm Cu	20.3	52.6	59.2	97.1	62.3
		n-Pentanal[f] (ppb)			
Control	57.4	69.9	107.4	26.2	51.2
5 ppm Cu	163.6	407.1	300.9	750.4	594.4
		n-Hexanal[f] (ppb)			
Control	10.5	60.7	67.4	20.5	44.0
5 ppm Cu	375.1	1234.8	1254.9	1763.3	1880.4

[a]Without copper exposure.

[b]With 5 ppm copper exposure at 4 C for 3 days.

[c]63 C, 30 min.

[d]Resuspended in boiled distilled water to the original volume of laboratory pasteurized milk.

[e]pH 6.7.

[f]Concentrations represent average of duplicate GLC analyses for each distillation.

Table 10. Concentrations of acetal, propanal, n-pentanal, and n-hexanal in whey fractions with and without fluorescent light exposure.

Treatments	Whey[c] fractions		
	Neutralized whey[d]	Dialyzed whey[e]	Dialysate
	Acetal[f] (ppb)		
0[a]	14.9	5.8	7.6
24h[b]	47.8	20.4	58.7
	Propanal[f] (ppb)		
0	12.6	3.4	8.0
24	19.8	8.0	10.6
	n-Pentanal (ppb)		
0	101.1	38.7	82.4
24	151.1	76.2	88.6
	n-Hexanal[f] (ppb)		
0	311.6	80.8	147.7
24	502.2	134.3	161.0

[a]Without light exposure.

[b]With F40CW light exposure at 4 C for 24 h.

[c]From laboratory pasteurized milk.

[d]pH 6.7.

[e]Dialyze 50 ml neutralized whey through cellophane membrane against 150 ml distilled water at 4 C with a mechanical stirrer for 48 h.

[f]Concentrations represent average of duplicate GLC analyses for each distillation.

Table 11. Concentrations of acetal, propanal, n-pentanal, and n-hexanal in whey fractions with and without copper exposure.

Treatments	Whey[c] fractions		
	Neutralized whey[a]	Dialyzed whey[e]	Dialysate
	Acetal[f] (ppb)		
Control[a]	14.9	5.8	7.6
5 ppm Cu[b]	64.2	51.4	42.3
	Propanal[f] (ppb)		
Control	12.6	3.4	8.0
5 ppm Cu	67.9	58.7	9.6
	n-Pentanal[f] (ppb)		
Control	101.1	38.7	88.6
5 ppm Cu	494.5	500.7	101.1
	n-Hexanal[f] (ppb)		
Control	311.6	80.8	147.7
5 ppm Cu	1539.2	1335.2	151.0

[a]Without copper exposure.

[b]With 5 ppm copper exposure at 4 C for 3 days.

[c]From laboratory pasteurized milk.

[d]pH 6.7.

[e]Dialyze 50 ml neutralized whey through cellophane membrane against 150 ml distilled water at 4 C with a magnetic stirrer for 48 h.

[f]Concentrations represent average of duplicate GLC analyses for each distillation.

Table 12. Concentrations of acetal, propanal, n-pentanal, and n-hexanal in a retail commercial skim milk fraction with and without fluorescent light exposure.

Treatments	Skim milk fractions					
	Skim milk	Neutralized[c] whey	Dialyzed[d] whey	Dialysate	Diluted[e] distillate	Pot[f] solution
			Acetal[g] (ppb)			
0[a]	24.0	16.7	5.8	36.8	27.7	2.1
24h[b]	66.0	67.9	18.6	60.6	49.6	20.4
			Propanal[g] (ppb)			
0	6.0	6.5	3.4	3.4	5.0	---
24	13.7	15.7	6.5	6.0	5.0	---
			n-Pentanal[g] (ppb)			
0	82.4	76.2	38.7	63.7	82.4	7.5
24	126.1	151.1	113.6	101.1	101.1	63.7
			n-Hexanal[g] (ppb)			
0	207.9	221.2	44.0	30.6	40.6	---
24	301.5	348.4	130.9	47.3	47.3	---

[a]Without light exposure.

[b]With F40CW light exposure at 4 C for 24 h.

[c]pH 6.7.

[d]Dialyze 50 ml neutralized whey through cellophane membrane against 150 ml distilled water at 4 C with a magnetic stirrer for 48 h.

[e]Distil 50 ml dialysate and collect 5 ml distillate, then dilute the distillate to 50 ml with boiled distilled water.

[f]The solution remaining in micro-kjeldahl flask after distillation of 50 ml dialysate to collect 5 ml distillate.

[g]Concentrations represent average of duplicate GLC analyses for each distillation.

Table 13. Concentrations of acetal, propanal, n-pentanal, and n-hexanal in a retail commercial skim milk fraction with and without copper exposure.

Treatments	Skim milk fractions					
	Skim milk	Neutralized whey[c]	Dialyzed whey[d]	Dialysate	Diluted distillate[e]	Pot solution[f]
			Acetal[g] (ppb)			
Control[a]	44.1	31.3	14.9	27.7	49.6	7.6
5 ppm Cu[b]	100.8	124.5	69.7	31.3	44.1	31.3
			Propanal[g] (ppb)			
Control	1.9	2.4	1.9	2.9	1.9	---
5 ppm Cu	7.5	10.6	8.0	5.5	6.5	6.0
			n-Pentanal[g] (ppb)			
Control	26.2	26.2	13.7	20.1	26.2	3.1
5 ppm Cu	163.6	201.0	107.4	32.4	38.7	10.3
			n-Hexanal[g] (ppb)			
Control	1.8	1.8	1.8	3.1	3.1	---
5 ppm Cu	33.9	47.3	27.2	13.9	10.3	20.5

[a]Without copper exposure.

[b]With 5 ppm copper exposure at 4 C for 3 days.

[c]pH 6.7.

[d]Dialyze 50 ml neutralized whey through cellophane membrane against 150 ml distilled water at 4 C with a magnetic stirrer for 48 h.

[e]Distil 50 ml dialysate and collect 5 ml distillate, then dilute the distillate to 50 ml with boiled distilled water.

[f]The solution remaining in micro-kjeldahl flask after distillation of 50 ml dialysate to collect 5 ml distillate.

[g]Concentrations represent average of duplicate GLC analyses for each distillation.

CHANGES IN CONCENTRATIONS OF SOME ALDEHYDES AFTER LIGHT EXPOSURE OR COPPER TREATMENT OF: A. MILK TREATED WITH SOME ANTIOXIDANTS, OR B. VARIOUS FRACTIONS OF MILK.

by

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This study was designed to determine: 1. the inihibitory effect of antioxidants in milk against light- and copper-induced oxidations. 2. the most susceptible fractions of laboratory pasteurized milk to light- and copper-induced changes. 3. the relative susceptibilities of milk neutralized whey, dialyzed whey, and dialysate from the same origin of laboratory pasteurized skim milk to light- and copper-induced changes. 4. the relative susceptibilities of neutralized whey, dialyzed whey, dialysate, diluted distillate, and distillation pot solution from the same origin of commercial skim milk to light- and copper-induced changes.

The TBA values of raw, laboratory pasteurized, and plant-pasteurized homogenized milks were 0.041, 0.045, 0.051, respectively after 30 min sunlight exposure; and 0.156, 0.207, 0.131, respectively after 5 ppm copper exposure for 3 days at 4 C. None of the samples would have been classified as oxidized according to their TBA tests with sunlight exposure. On the contrary, all of the milk samples showed certain degree of lipid oxidation according to their TBA values with copper exposure.

Na_2SO_3 (10^{-3}M) reduced acetal, propanal and n-pentanal concentrations but did not reduce the concentration of n-hexanal (30.4 ppb) after 12 h fluorescent light exposure in the display case. Control milk without light exposure but with or without added Na_2SO_3 contained 0 ppb n-hexanal as analyzed by GLC. The most effective combination of antioxidants against milk exposed to sunlight was BHA + PG + sodium citrate. This mixture reduced the concentration of acetal, propanal, n-pentanal, and n-hexanal

in light-exposed milk from 18.6, 6.5, 251.0, and 87.4 ppb to 7.1, 2.7, 69.9, and 10.5 ppb, respectively. On the other hand, BHA + EDTA was most effective against copper (5 ppm) induced oxidation and reduced the concentrations of the same aldehydes from 46.0, 57.7, 644.3, and 1351.9 ppb to 7.6, 1.9, 32.4, and 7.2 ppb, respectively.

Neutralized whey was more susceptible than resuspended cream from the same laboratory pasteurized milk origin to light-induced changes. The concentration ratios of C_2, C_3, C_5 and C_6 aldehydes of with/without fluorescent light exposure for 51 h at 4 C were 4.8, 6.5, 6.2 and 47.3 for neutralized whey, while 2.4, 5.4, 4.7, and 12.9 for resuspended cream, respectively. On the contrary, the neutralized whey was less susceptible than resuspended cream to copper-induced changes. The concentration ratios of C_2, C_3, C_5 and C_6 aldehydes of with/without 5 ppm copper exposure for 3 days at 4 C were 2.2, 12.5, 11.6 and 42.7 for neutralized whey, while 14.6, 33.5, 28.6 and 86.0 for resuspended cream, respectively. Except the concentration ratio of acetal (3.5 for dialyzed whey and 7.7 for dialysate) of with/without 24 h fluorescent light exposure at 4 C dialyzed whey seemed to contribute more than the dialysate to light-induced changes.

From a retail commercial skim milk, there were dialysable but not distillable precursors of the four carbonyl compounds analyzed in the distillation-pot solution. The concentration ratios of acetal and n-pentanal from distillation-pot solution

of with/without 24 h fluorescent light exposure at 4 C were 9.7 and 8.5, while those of with/without 5 ppm copper exposure for 3 days at 4 C were 4.1 and 3.3