

**THE SENSORY AND ANALYTICAL ANALYSES OF NONFAT
MILK FORMULATIONS: STABILITY TO LIGHT OXIDATION
AND PASTEURIZATION**

BY
JODI POWELL

Thesis submitted to the Faculty of Virginia Polytechnic Institute and State University in
partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE
IN FOOD SCIENCE AND TECHNOLOGY
APPROVED BY:

Susan E. Duncan, Chair

Sean O'Keefe

Susan S. Sumner

THE SENSORY AND ANALYTICAL ANALYSES OF NONFAT MILK
FORMULATIONS: STABILITY TO LIGHT OXIDATION AND PASTEURIZATION

By

Jodi Powell

Susan E. Duncan, Chair

Department of Food Science and Technology, Virginia Polytechnic Institute and State
University

ABSTRACT

Sweet cream liquid buttermilk and skimmed milk ingredients were heat processed and/or exposed to fluorescent light to determine changes in potential flavor compounds. Solid phase microextraction-gas chromatography/flame ionization detection was used to analyze the concentrations of the volatile compounds (2-butanone, 2-pentanone, acetaldehyde, diacetyl, hexanal, methyl sulfide) found in the two components.

Pasteurized unoxidized skimmed milk had measurable levels of 2-butanone, acetaldehyde, and diacetyl. Pasteurization of skimmed milk increased concentration of 2-pentanone and methyl sulfide to measurable levels. However only 2-butanone and acetaldehyde were detectable in oxidized skimmed milk. All liquid buttermilk ingredient treatments had measurable concentrations of 2-butanone, 2-pentanone, and acetaldehyde. Pasteurization of unoxidized liquid buttermilk increased the concentration of diacetyl and hexanal to measurable levels whereas oxidized buttermilk, both pasteurized and unpasteurized, had measurable levels of hexanal and methyl sulfide.

Nonfat (.3%) dairy beverages were formulated using the same components to determine if the volatiles in liquid buttermilk might enhance the flavor of nonfat milk. Triangle tests and hedonic tests were performed on the nonfat formulations to determine

their overall difference and overall acceptance. There was no significant difference between formulations.

ACKNOWLEDGEMENTS

I would like to thank Dr. Susan Duncan for her support and friendship throughout this program, and for exposing me to things that I may never have been exposed to while in this department. I would also like to thank Dr. Randy Grayson and Dr. Larry Moore in the Minority Academic Opportunities Program (MAOP) office for financing my masters program along with Dr. Duncan. I would also like to thank them for all the moral support and outreach that was offered through their office. I would also like to thank Ms. Lindell Williams, Dr. Henry Bahn, and all the staff at USDA/CSREES/SERD-HEP office for supporting me throughout my B.S. and M.S. degrees with very flexible internships in the summer months and on holidays. I would like to thank Mr. Walter Hartman and Mrs. Kim Waterman for all their support and help with the processing and keeping me focused. I would also like to thank Harriet Williams for her help with the Gas Chromatography and Dr. Sean O'Keefe for all his guidance and support using the Solid Phase Microextraction/Gas Chromatography technique. I would like to thank Dr. Susan Sumner for her help and guidance as a committee member throughout the process. I would like to thank my office mates, Sonia Gonzales for all the sensory help, Marleen Van Aardt for the GC help, Cole Bolling, and Jennifer Goode for their support and help with the entire graduate school process, and the research.

DEDICATION

I dedicate this work to my father and mother, Adrian and Christine Powell, who have offered me an unimaginable amount of love and support throughout this entire process. Without them, I never would have reached this goal, nor the many more to come. I would also like to dedicate this to my brother, Adrian Powell II and my niece and god-daughter Jaelyn Jodi Powell (Jae-Jae) who always keep me inspired when I am down. My friends Jocelyn Smith, Ngozi Ogbuawa, Cicely Washington and Jason Williams who would call and visit Blacksburg, VA to ensure that I was okay, I will never forget your friendship and love. And last but not least, I would like to dedicate this to my roommate, Nonye Onyewu who kept me focused, laughing and well fed. You will never know how much I appreciate and love you. Thank you so much, and I wish you the best.

TABLE OF CONTENTS

TITLE		i
ABSTRACT		ii
ACKNOWLEDGEMENTS		iv
DEDICATION		v
TABLE OF CONTENTS		vi
LIST OF TABLES		viii
LIST OF FIGURES		ix
CHAPTER I.	REVIEW OF LITERATURE	1
	SENSORY CHARACTERISTICS OF MILK	1
	SWEET CREAM BUTTERMILK	5
	LIGHT OXIDATION	6
	PASTEURIZATION	9
	SOLID PHASE MICROEXTRACTION	11
	REFERENCES	13
CHAPTER II	THE SENSORY AND ANALYTICAL ANALYSIS OF LOWFAT MILK FORMULATIONS: STABILITY TO LIGHT OXIDATION AND PASTEURIZATION	
	INTRODUCTION	15
	MATERIALS AND METHODS	18
PART I.	Evaluation of Flavor Compounds of Dairy Components	18
	Processing of Raw Milk into Dairy Ingredients	18
	Light Oxidation of Pasteurized and Unpasteurized Dairy Components	18
	Composition of Dairy Ingredients	20
	Analysis of Volatile Flavor Components	20
PART II.	Improving the Sensory Characteristics of Nonfat Milk by Adding Sweet Cream Buttermilk	21
	Sensory Analysis	22
	Statistical Design	23
	RESULTS AND DISCUSSION	24
PART I.	Evaluation of Flavor Volatiles of Dairy Components	24
	Gross Composition and Color of Components	24
	Volatile Flavor Analysis of Components	26

PART II.	Improving the Sensory Characteristics of Nonfat Milk by Adding Sweet Buttermilk	31
	Preliminary Data on Dairy Components for Nonfat Milk Beverages	32
	Composition and Color of Milk Beverages Using Skimmed Milk or Liquid Buttermilk Components	32
	Sensory Results for Formulations	33
	CONCLUSIONS	35
	REFERENCES	36
CHAPTER III.	APPENDIX	38
	Sensory Information	39
	Triangle Test Score Card	39
	Hedonic Test Score Card	40
	Human Subjects Form	41
VITA		42

LIST OF TABLES

Table 1. Key volatiles detected by dynamic headspace at varying fat levels.	8
Table 2. Gross composition of pasteurized or unpasteurized components with or without light exposure.	24
Table 3. L*, a*, and b* values for skimmed milk and liquid buttermilk.	25
Table 4. Volatile flavor concentrations (ppb) in skimmed milk and liquid buttermilk components.	30
Table 5. Preliminary data for dairy beverages using liquid buttermilk, nonfat dry milk, dry buttermilk and skim milk.	32
Table 6. Proximate analysis of fat, protein, moisture and ash of the nonfat (.3%) milk formulations using liquid buttermilk or skimmed milk components.	32
Table 7. Color values (L*, a*, and b*) ¹ for pasteurized nonfat milk beverages formulated with liquid buttermilk or cream with skimmed milk with or without light exposure.	33

LIST OF FIGURES

- Figure 1. Riboflavin (sensitizer) is activated by light (hv) that then reacts with oxygen. The oxygen (O₂) is then activated by the excited riboflavin (sen*). This activated oxygen (¹O₂) then reacts with the fatty acids (RH) and gives fatty acid peroxides (ROOH) (DeMan, 1999). 7
- Figure 2. Flow chart of pasteurization and light oxidation of dairy ingredients. 19
- Figure 3. Milk samples were spiked with 50, 100, 200, and 400 µm samples to make 50, 100, 200, and 400 ppb concentrations of hexanal (.965), 2-pentanone (.964), methyl sulfide (.963), 2-butanone (.959), diacetyl (.930), and acetaldehyde (.905). The R² values were calculated to determine accuracy and precision of technique. The graph is plotted Area of analyte/Area Internal Standard vs. Weight of analyte/Weight of Internal Standard. 27
- Figure 4. Gas chromatogram of pasteurized and oxidized liquid buttermilk with 4-methyl-2-pentanone as the internal standard. Acetaldehyde, dimethyl sulfide, 2-pentanone, and hexanal were identified using retention times of standards. 29
- Figure 5. Gas chromatogram of unpasteurized and oxidized skim with 4-methyl-2-pentanone as the internal standard. Acetaldehyde and 2-butanone were identified using retention times of standards. 29
- Figure 6. The hedonic scores for the oxidized and unoxidized milk beverages formulated with 75% liquid buttermilk and 25% skimmed milk (Buttermilk), and hedonic scores for the oxidized and unoxidized milk beverages formulated with 99% skimmed milk and 1% cream (Skim) for 2 replications were not significantly different. 34

CHAPTER I

LITERATURE REVIEW

SENSORY CHARACTERISTICS OF MILK

Whole and nonfat milk can be characterized by three characteristics: appearance, texture, and flavor. Appearance is characterized as whiteness, glossiness, and transparency of the liquid milk (Phillips et al., 1995a). Saba et al. (1998) used the same descriptors but also included the term yellowness. Flavor descriptors include astringent, bitter, cooked, oxidized, salty, buttery, and sweet (Phillips et al., 1995a; Saba et al., 1998). Texture would be the measure of viscosity or mouth feel of the sample. Descriptors applied to texture characteristics included fat, heavy, watery and light (Saba et al., 1998).

Appearance of whole milk and lowfat milk varies greatly due to differences in fat concentration. Whole milk contains not less than 3.25% milk fat and 8.25% solids-not-fat. Lowfat milk contains .5, 1.5, or 2.0% milk fat and not less than 8.25% solids-not-fat. Skim or nonfat milks contain less than .5% milk fat and 8.25% solids not-fat (Miller, 2000). Light scattering by fat globules and casein micelles causes milk to appear opaque. Skim milk appears slightly blue because casein micelles scatter the shorter wavelengths of visible light (Walstra and Jenness, 1984). A carotenoid precursor of vitamin A, β -carotene, contained in milk fat is responsible for the creamy milk color, and riboflavin adds a greenish color (Hui, 1993). The milk fat globule membrane (MFGM) also has casein micelles emulsifying proteins that reflect light and contribute to the white appearance of milk.

Phillips et al. (1995a) measured the white (L), green (a) and blue (b) values of 2% milk formulated with cream and 2% milk formulated with nonfat dry milk and cream using a Macbeth Color-Eye Spectrophotometer. The nonfat dry milk formulated with cream had an average L value of 75.55 , whereas the L value for the milk formulated with fat was at 81.11 (Phillips et al., 1995a). The higher L value indicates whiter appearing milk.

In milk, the fat globules and casein micelles are primarily responsible for scattering light. This light scattering is caused by particles of larger than molecular dimensions whose refractive index differs from that of the surrounding medium (Hui, 1993). The larger molecules in milk are the milk fat globule membrane and the casein micelles, whereas the effect of serum protein molecules and somatic cells is almost negligible, with respect to light scattering, because of the relatively small size and low concentrations respectively of the structures (Hui, 1993). Casein micelles are smaller than fat globules and scatter less light thereby giving the blue color in skim and lowfat milk with lower concentrations of fat than whole milk.

Processing also has effects on the appearance of milk. Homogenization causes the diffuse reflection of light to increase, making the milk appear whiter (Walstra and Jenness, 1984). Heat treatment of milk also causes color changes. At first, the milk becomes slightly whiter due to the denaturation of proteins. More intense heating then causes Maillard reactions to occur, with the characteristic browning reaction, resulting in brown pigments (Walstra and Jenness, 1984).

Viscosity and mouthfeel describe the texture of milk. The viscosity of milk is a vital characteristic with regards to overall satisfaction (Mela, 1988). Whole milk has a

creamy and heavy mouthfeel while lowfat milks usually have a lighter and watery consistency (Saba et al., 1998). These differences in texture are caused by the milk fat or lack thereof (Phillips et al., 1995b). The MFGM has emulsifying characteristics due to the physical properties of both the proteins and the lipids that are attached to it. In raw milk, the membrane acts to protect the milk fat from destructive reactions such as the action of lipase enzymes which may produce hydrolytic rancidity. However, during homogenization and separation, this membrane is altered and destroyed decreasing the surface area lipase requires as a fat-water surface for activity. All of these functional properties are lost with the removal of the milk fat globule membrane with the milk fat during the separation stages of the milk process. Therefore, lowfat milks do not have the creamy and thick texture evident in whole milk as described previously (Saba et al., 1998; Phillips et al., 1995b).

Flavor is also an important sensory characteristic. Nonfat milk drinkers recognize that nonfat and lowfat milk products are less satisfying and versatile than whole milk, but are willing to sacrifice this lack of flavor for the health and nutritional benefits that come along with lower fat consumption (Miles et al., 1995). Fat enhances the flavor of whole milk by contributing naturally occurring flavor compounds that are removed from milk when fat is removed (Phillips et al., 1995b). Flavor descriptors used to describe lowfat milk include astringent, bitter, and cooked (Phillips et al., 1995b). Whole milk descriptors include buttery, sweet, salty and oxidized. The sweet and salty flavor comes from naturally occurring sugars and salts in milk such as lactones and minerals. Various aldehydes contribute to an oxidized flavor. Astringent and bitter flavors result from the

nitrogen and sulfur compounds derived from the enzymatic breakdown of carbohydrates and protein compounds.

Numerous chemical compounds are involved in creating these flavor attributes, particularly off-flavors. Aldehydes, such as acetal, propanal, n-pentanal, hexanal, and other flavor compounds, including alkanes, lactones, esters, sulfur compounds, nitrogen compounds, aliphatic compounds and other aromatic hydrocarbons, occur naturally in the milk or are caused by the spontaneous oxidation or light oxidation of milk during storage (Whaemi et al., 1988). Proteins also have an impact on flavor of the milk. Enzymes such as proteases and lipases affect the flavor and stability of milk (Varnam and Sutherland, 1994).

There have been several technological approaches to enhancing the sensory characterization of lowfat milk through formulations. These attempts have been made by using fat substitutes such as Litesse (Cultor, New York, NY) and Dairy Lo (Cultor, New York, NY), commercial fat substitutes, and also by formulating milk samples with nonfat dry milk (NDM) (Phillips and Barbano, 1997). When both NDM and fat substitutes were added to lowfat (2%) milk, samples were whiter and more viscous than lowfat milks without added ingredients based on sensory and analytical tests. Viscosity was increased also when NDM and fat substitutes were added. Viscosity was measured using an Ostwald-type viscometer. However, viscosity was not measurably changed as evaluated by a trained sensory test panel (Phillips et al., 1995b; Phillips and Barbano, 1997). Formulations with buttermilk powders also have been developed for recombined milk products. These products tend to have a more complete fresh milk flavor than milks formulated with NDM or butter oil powders. A sensory panel reported that 60% of panel

members preferred recombined milk with 10% of the NDM replaced with BMP more so than the fresh milk samples (Newstead, 1999). This indication was matched by a 500% increase in sales when the recombined milk formulation was changed to BMP (Newstead, 1999).

Alternate processing techniques also have been used to enhance the sensory properties of lowfat milks. Ultrafiltration is used to enhance the texture of lowfat milk by filtering water and thereby having more milk solids in the beverage for better flavor and texture.

SWEET CREAM BUTTERMILK

Sweet cream buttermilk, and butter-derived aqueous phase, which have a high concentration of milk fat globule membrane (MFGM) as well as flavor compounds that can be used to enhance the flavor of lowfat milk without adding as much fat as cream. During the churning process the MFGM emulsion breaks and MFGM is released into the sweet cream buttermilk phase. Buttermilk contains both lipid and protein components that are not found in high concentrations in other milk fractions. The flavor of fresh sweet buttermilk is typically milky, sweet and buttery (Heiler and Schieberle, 1996). The composition is similar to skim milk except for the concentration of molecules associated with the MFGM, including specific proteins and phospholipids.

The protein composition of sweet buttermilk is 28.35mg/g protein (Elling et al., 1996). Butyrophilin constitutes more than 40% of total protein associated with MFGM. PAS-6 and PAS-7 are the most abundant glycoproteins in bovine MFGM after butyrophilin (Corredig and Dalgleish, 1998). Serum proteins, which include the caseins

and whey proteins, β -lactoglobulin and α -lactalbumin, constitute about 75% of the total protein in milk.

Previous data suggests that off-flavors that occur in buttermilk come from the MFGM because the off-flavors that occur in sweet buttermilk do not occur in nonfat milks (Heiler and Schieberle 1996). Buttermilk develops many off flavors caused by the autoxidation of the unsaturated fatty acids such as oleic, linoleic, and linolenic acid incorporated in the buttermilk (Swoboda, 1977). By using column chromatography on silica gel to analyze flavor compounds associated with off-flavor, 13 compounds that contribute to off-flavors were identified in cultured sweet cream buttermilk: eight of these same compounds were found in cultured sour cream buttermilk and fresh butter oils. These compounds were σ -decalactone, (E,Z)-2,6-nonadienal, 3-methyl-indol, σ - and γ -octalactone, diacetyl, vanillin, 4,5-epoxy – (E)-2-decanal. These compounds are mostly products of autoxidation of lipids (Heiler and Schieberle 1996).

Autoxidation in sweet buttermilk is potentiated by the large concentration of unsaturated fatty acids contained in sweet buttermilk. Most of these unsaturated fatty acids are associated with the MFGM. Autoxidation of these fatty acids in the MFGM creates the unsavory oxidized flavor that is not found in skim milk.

LIGHT OXIDATION

Although autoxidation has been shown to occur in milk, light oxidation is also a major factor in promoting the development of off-flavors in milk. Light oxidation is the catalytic effect of light in promoting the off-flavor development in milk. The extent of light oxidation has three major variables: the length of exposure, the intensity of exposure, and the wavelength of the light exposure (DeMan, 1999). Two major flavors

are developed from light oxidation: burnt/activated, which is the sunlight flavor and, after longer exposure, the characteristic oxidized flavor (DeMan, 1999).

Riboflavin plays a significant role in the oxidation process as a photosensitizer (DeMan, 1999). Photodegradation of riboflavin in milk was found to be primarily dependent upon wavelength, intensity of light, exposure time, protective effect of the packaging material, and temperature at which the milk is stored. Riboflavin is transferred into its excited state by light. This activated compound then reacts with oxygen to form a singlet oxygen that is free to react with fatty acids and produce peroxides (DeMan, 1999). The singlet oxygen reacts directly with the double bond of the unsaturated fatty acids by addition, and shifts the double bond one carbon away. The peroxides further break down into aldehydes and ketones to form the unpleasant flavor that is characteristic of oxidation.

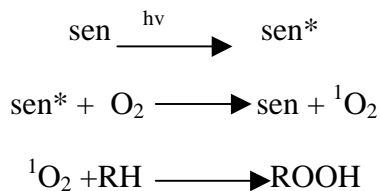


Figure 1. Riboflavin (sensitizer) is activated by light (hv) that then reacts with oxygen. The oxygen (O₂) is then activated by the excited riboflavin (sen*). This activated oxygen (¹O₂) then reacts with the fatty acids (RH) and gives fatty acid peroxides (ROOH) (DeMan, 1999).

During storage of foods, a number of chemical changes can occur involving not only lipids, but also proteins. Light-induced oxidation of proteins has been shown to lead to off-flavors and destruction of essential amino acids in milk. Sunlight with wavelengths between 400 and 800 nm attacks methionine and converts it into methional which can cause a typical sunlight off-flavor at concentrations as low as .1 ppm (Patton, 1954). There is also an interaction between the free radicals formed by lipid oxidation

and proteins which leads to oxidation off-flavors. Methionine can react with lipid peroxides to yield methionine sulfoxide.

Using vacuum distillation-solvent extraction (VDSE), gas chromatography-olfactometry (GC-O), static headspace gas chromatography (SHGC), and dynamic headspace gas chromatography (DHGC), Cadwallader and Howard (1998) determined several aldehydes that contribute to the light activated flavor of the milk. Some of the identified key volatiles were methanethiol, acetaldehyde, dimethyl sulfide, 2,3-butanedione, pentanal, 1-hexene-3-one, hexanal, 1-octen-3-one, octanal, and 1-nonen-3-one.

Cadwallader and Howard (1998) reported that, based on sensory evaluation, light-activated flavor in milk is impacted by the fat level of the milk. Whole milk had an increase in light-activated flavor, mushroom flavor, and butterscotch flavor. Skim and 2% milk had higher plastic flavor concentrations. Creamy and buttery flavors were increased also in whole milk and 2% milk. These results also show the differences in the concentration of the key volatiles at varying fat levels (Table 1).

Table 1. Key volatiles detected by dynamic headspace at varying fat levels.

Compound	Skim	2%	Whole
Acetaldehyde	nd/+	+ / ++	nd / +
Dimethyl sulfide	+ / ++	++ / ++	++ / ++
2,3-butanedione	nd / nd	nd / +	nd / +
Hexanal	nd / ++	nd / ++	nd / ++
Octanal	+ / +	+ / +	nd / +

Nd=not detected, +=weak, ++=intermediate; control/light activated milk (Cadwallader and Howard, 1998)

These compounds, depending upon concentration all have significant flavor descriptors. Acetaldehyde has a green apple taste; dimethyl sulfide has a creamed corn taste; 2,3-butanedione has a buttery taste; hexanal tastes grassy; and octanal tastes like oranges (Cadwallader and Howard, 1998).

Marsili (1999) determined that there is an overall increase in pentanal and hexanal in 2% milk and skim milk after light oxidation by using solid phase microextraction-gas chromatography-mass spectrometry (SPME-GC/MS) and dynamic headspace gas chromatography-mass spectrometry (DHGC-MS). Marsili (1999) also tried to determine if a significant difference could be determined through sensory means to analyze the increasing concentrations of pentanal and hexanal due to light oxidation, but no significant difference could be found.

Bassette et al. (1983) using head space gas liquid chromatography found that the concentrations of acetaldehyde, propanal, n-pentanal, and n-hexanal all increased over three days with the light treated whole milk and lowfat milk samples as opposed to samples stored for three days in the dark. A hedonic sensory test showed that the highest scores were given to the milk stored in the dark for three days, and the scores progressively decreased for the samples stored in the light for three days.

PASTEURIZATION

Pasteurization is the process applied to a product with the object of minimizing possible health hazards arising from pathogenic microorganisms. With milk this is done by heat treatment, which is consistent with the minimal chemical, physical, and organoleptic changes in the product. Fluid milk is pasteurized by heating under various time and temperature conditions. There are several different combinations and methods, high temperature short time (HTST), ultra high temperature (UHT), and batch pasteurization. The most popular commercial pasteurization method is HTST which has the least detrimental effect to the product. UHT processing gives milk a very cooked flavor that is caused by the break down of sulfur groups in whey proteins.

Pasteurization also leads to the increased susceptibility of milk to light oxidation. Bassette et al. (1983) discussed the effect of the pasteurization temperature on the susceptibility of milk to light oxidation by examining the increase or decrease in four volatile carbonyls which reflect both light oxidation and pasteurization. Concentrations of acetaldehyde, propanal, n-pentanal, and n-hexanal increased much more in the light treated samples than those kept in the dark (Bassette et al., 1983) when analyzed by gas-liquid chromatography. However, high-heat treatment (90°C) lessened those increases in propanal and n-hexanal but enhanced increases in acetaldehyde and n-pentanal (Bassette et al., 1983). High heat treatment also showed a significant decrease in the oxidized flavor of the samples, but this has been shown to be due to the increase in the acetaldehyde and propanal which contributes to a more cooked flavor that masked the oxidized flavor.

Senorans et al. (1996) used a programmed temperature vaporizer (PTV)-gas chromatography-mass spectrometry (GC-MS) to analyze aldehyde and ketone concentrations of raw and pasteurized milk. Using PTV GC-MS, Senorans et al. (1996) identified acetone, ethyl acetate, butanone, 2-pentanone, phenyl acetaldehyde, 2-heptanone, 2-nonanone, acetic acid, benzaldehyde, ethyl benzaldehyde and hexanal in raw milk. In pasteurized milk, the same flavor compounds were found, but the 2-heptanone concentration was most affected by the heat treatment. Calculated by matrix of peak areas, in the raw milk the concentration of 2-heptanone was 0.0 and after regular pasteurization, the matrix peak area increased to 7.5. This demonstrates the increase in 2-heptanone by the pasteurization.

SOLID PHASE MICROEXTRACTION

Solid phase microextraction (SPME) is a solvent free technique used for sample preparation that can integrate sampling, extraction, concentration and sample introduction into a single step (Zhang et al., 1994). SPME consists of two processes: partitioning of analytes between the coating and the sample and desorption of concentrated analytes into an analytical instrument. In the first process, the coated fiber is exposed to the sample and the target analytes are extracted from the sample matrix into the coating. The fiber with the concentrated analytes is then placed into the instrument for desorption, separation, and then quantitation.

The fiber is a fused silica coated with a gas chromatographic stationary phase. The small size of the fiber allows it to be incorporated into a syringe like device, which can then be operated like an ordinary syringe. Extraction techniques from the matrix are used based on their sensitivity. Several factors that can influence the sensitivity of the fiber are the volume of fiber-coating, the fiber-coating characteristics, derivatization of target analytes, modification of matrices, heating the sample and cooling the coating (Zhang et al., 1994). The proper coating is determined by the polarity of the compounds that is not to be extracted. Because both the sample matrix and the coating compete for the target compound, the affinity of the coating for target analytes is crucial in SPME sampling. Due to the partition coefficients of analytes being partially determined by the interaction between the target compound and the sample matrix, the nature of the matrix can be modified by adding salt or heating the sample. Adding salt to aqueous samples increases the ionic strength thereby increasing the partitioning of polar organic compounds into the polymer coating (Zhang et al., 1994). For thermally stable analytes,

heating the sample is a convenient way to release analytes from the matrix into the headspace and improve sensitivity.

SPME is a sensitive, rapid procedure for testing lipid photooxidation products in milk. Marsili (1999) discussed the comparison between dynamic headspace (DH) and solid phase microextraction and found that solid phase microextraction is more precise, more sensitive, and has a lower coefficient of variation between replicates.

Commercially available DH equipment is more expensive than SPME equipment, but does not appear to offer significant advantages with respect to improved sensitivity.

SPME has been shown to be a more desirable sample preparation technique than DH with none of the problems with carryover, background or ghost peaks that occurs with the DH equipment. Marsilli (1999) showed that SPME is a viable substitute for DH for studying oxidation off flavors in milk.

REFERENCES

- Bassette, R., Fung, D.Y.C., and Roberts, H. 1983. Effect of pasteurization temperature on susceptibility of milk to light-induced flavor. *J. Food Prot.* 46:416-419.
- Cadwallader, K. and Howard C.L.,. Analysis of aroma active components of light activated milk. In *Flavor Analysis: Development in Isolation and Characterization*, M. Morrello and C. Mussinan, Eds. ACS Symposium Series N. 705, American Chemical Society, Washington, DC, 1998. pp 343-358.
- Corredig, M. and Dalglish, D. 1998. Buttermilk properties in emulsions with soybean oil as affected by fat globule membrane derived proteins. *J. Food Sci.* 63(3):476-480.
- DeMan, John. 1999. *Principles of Food Chemistry*, 3rd Ed. Aspen Publishing Company. Gaithersburg, MD.
- Elling, J.L., Duncan, S.E., Keenan, T.W., Eigel, W.N., and Boling, J. 1996. Composition and microscopy of reformulated creams from reduced cholesterol butteroil. *J. Food Sci.* 61(1):48-52.
- Heiler, C. and Schieberle, P. 1996. Studies on the metallic off flavor in buttermilk: Identification of potent aroma compounds. *Lebensm-wiss-u-Technol.*29:460-464.
- Hui, Y.H. 1993. *Dairy Science and Technology Handbook*. VCH Publishers, Inc. New York, NY.
- Marsili. R.T. 1999. Comparison of solid phase microextraction and dynamic headspace Methods for the gas chromatographic mass spectrometric analysis of light induced lipid oxidation products in milk. *J. Chromat. Sci.* 37:17-23.
- Mela, D. 1988. Sensory assessment of fat content in fluid dairy products. *Appetite*10:37-44.
- Miles, H., Schwager, S., and Lenz S. 1995. Perceptual dimensions that influence consumers' choice of milk type for beverage use. *Agribusiness* 11:(3)263-272.
- Miller, Gregory. 2000. *Handbook of Dairy Chemistry*, CRC Press, Inc., Boca Raton.
- Newstead, D. F. Sweet-cream buttermilk powders: Key functional ingredients for recombined milk products. In *Recombined Milk and Milk Products*. pp. 55-60, IDF Special Issue 9902. International Dairy Federation, Brussels, Belgium.
- Patton, S. 1954. Browning and associated changes in milk and its products. A review. *J.Dairy Sci.* 38:457.

- Phillips, L. and Barbano, D. 1997. The influence of fat substitutes based on protein and titanium dioxide on the sensory properties of lowfat milks. *J. Dairy Sci.* 80:2726-2731.
- Phillips, L., McGiff, M.L., Barbano, D., and Lawless, H. 1995a. Influence of nonfat dry milk on the sensory properties of viscosity and color of lowfat milks. *J. Dairy Sci.* 78: 2113-2118.
- Phillips, L. McGiff, M., Barbano, D., and Lawless, T. 1995b. The influence of fat on the sensory properties, viscosity and color of lowfat milk. *J. Dairy Sci.* 78:258-1266.
- Saba, A., Moneta, E., Nardo, N., and Sinesio, F. 1998. Attitudes, habit, sensory, and liking expectations as determinants of the consumption of milk. *Food Quality and Preference.* 9(1/2):31-41.
- Senorans, F.J., Tabera, J. Herraiz, M., and Reglero, G. 1996. A method for the direct isolation and gas chromatographic analysis of milk flavor components using a programmed temperature vaporizer. *J. Dairy Sci.* 79:1706-1712.
- Swoboda, P. 1977. Metallic odor caused by vinyl ketones formed in the oxidation of butterfat: the identification of octa-1-cis-5-diene-3-one. *J. Sci., Food and Ag.* 28:1019-1024.
- Varnam and Sutherland. 1994. *Milk and Milk Products: Technology, Chemistry, Microbiology.* Chapman and Hall, London.
- Walstra, P. and Jenness, R. 1984. *Dairy Chemistry and Physics.* Wiley and Sons, New York.
- Whaemei, J., Bassette, R., and Crang, R. 1988. Effects of light and copper on volatile aldehydes of milk and milk fractions. *J. Dairy Sci.* 71:2366-2372.
- Zhang, Z., Yang, M., and Pawliszyn, J. 1994. Solid phase microextraction. *Analyt. Chem.* 66(17):844-853.

CHAPTER II

THE SENSORY AND ANALYTICAL ANALYSIS OF NONFAT MILK FORMULATIONS: STABILITY TO LIGHT OXIDATION AND PASTEURIZATION

INTRODUCTION

The flavor of milk is influenced primarily by the interaction of milk components during processing and storage. The amount of fat and the balance of proteins and total solids not only affect the flavor of the milk, but also the texture and color. Flavor, texture, and color are all factors involved in consumer choice and acceptance of nonfat milk. Researchers have increased total solids to enhance the color of skim milk by adding nonfat dry milk and fat substitutes (Phillips and Barbano, 1997), by ultrafiltration to also increase total solids, and by adding buttermilk powder to increase milkfat and total solids thereby enhancing texture, flavor and functionality (Newstead, 1999). Skimmed milk with added buttermilk powder was shown to have better flavor than nonfat milk alone and was preferred by consumers (Norman, 1955). No reports have shown how fresh liquid buttermilk would affect volatile flavor compounds developed during light exposure and pasteurization, or how these processes would affect flavor, color and consumer acceptability.

Flavor of milk can be impacted by a variety of external and intrinsic factors. Light oxidation has been shown to be detrimental to the flavor of milk giving it a burnt and “activated” flavor caused by the breakdown of fatty acids. The breakdown of these fatty acids yields various aldehydes and ketones that have been identified as causes of various off-flavors in the milk. Using different analytical techniques, Cadwallader and Howard (1998) identified several key compounds that are specific to light oxidation in

milk. Pasteurization also has been shown to increase the off-flavors of milk giving it a very cooked flavor (Bassette et al., 1983).

Light oxidation has been shown to give very distinct flavors that are caused by aldehydes and ketones and their combinations. Descriptive flavor profiling was done on whole, 2% and skim milk by Cadwallader and Howard (1998) to determine the description of the off flavors before, during and after 18 hours of light oxidation. This was done in conjunction with analytical techniques to demonstrate that the changes in concentration of the aldehydes during light oxidation were directly related to off-flavors noticed by the sensory panel.

The sensory attributes and health issues can be addressed to create both a healthy and palatable milk product. Ultrafiltration of the lowfat milk has an influence on viscosity along with pasteurization temperature and time (Newstead, 1999). Ultrafiltration also increases total milk solids, proteins and vitamins that are sometimes lost in nonfat milk products. Formulated milk beverages also may enhance sensory characteristics and increase potential for additional health benefits. Use of underutilized milk components with concentrated levels of CLA, such as liquid buttermilk, may allow milk to function as a nutraceutical. Increased CLA intake along with other micronutrients and phospholipids that are concentrated in the MFGM, available in ingredients such as fluid buttermilk, help to position nonfat milk formulations as healthy and flavorful alternatives to other beverages.

The first component of this project was to evaluate the volatile flavor chemistry of two dairy ingredients: liquid buttermilk and skimmed milk. A comparison of the volatile chemistry before and after light oxidative and pasteurization conditions of these

ingredients were made quantitatively and qualitatively. The objective of the first component was to identify key volatile flavor components in these dairy ingredients and determine any changes to due to light oxidation and/or pasteurization.

The second component of the project involved formulating a nonfat (.3%) dairy beverage utilizing an underutilized dairy product, sweet cream buttermilk, and to evaluate light oxidative stability of the formulated beverage. A sensory panel evaluated the formulations for overall difference. The objective of this component was to enhance the flavor of nonfat milk products using a nutrient rich underutilized dairy ingredient.

MATERIALS AND METHODS

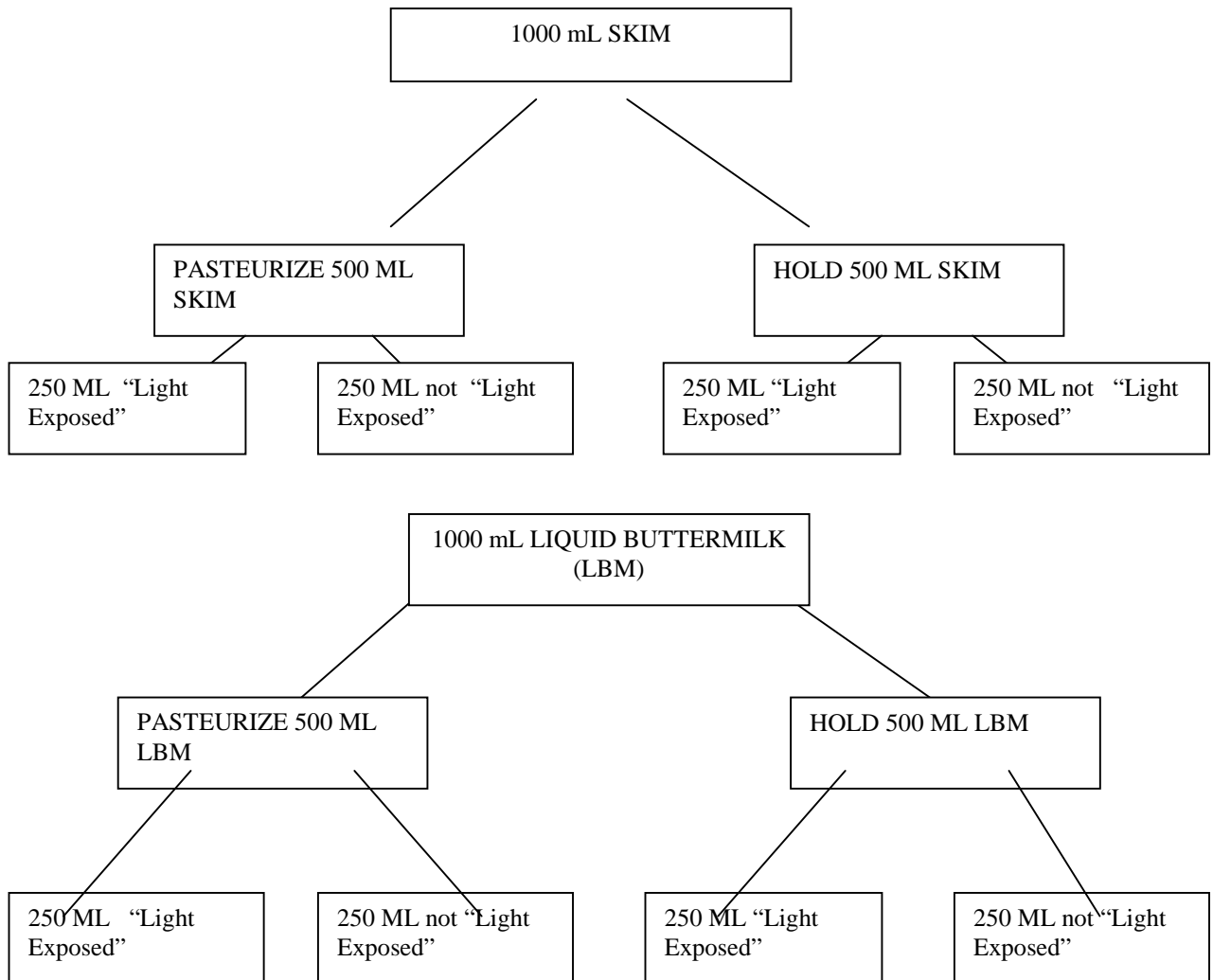
Part I. Evaluation of Flavor Components of Dairy Ingredients

Processing of Milk into Dairy Ingredients. Raw milk was obtained from the Virginia Tech dairy farm and separated into cream and skim phases using a pilot plant separator (Elecrem separator, model IG, 6400 rpm, Bonanza Industries, Inc., Calgary, Alberta). The skim phase was cooled and stored at 4-7°C. The cream was vat pasteurized for 30 min at 68.3°C, and then cooled. Cream was tempered at 13-14°C and incubated overnight. Sweet buttermilk and butter were made by mechanically churning the cream in a glass jar at 16.7°C. Buttermilk was separated from butter granules by pouring it through cheesecloth and pressing butter granules with a stainless steel spoon to remove excess buttermilk from the butter. A portion of the skim phase and sweet buttermilk was retained as “unpasteurized” dairy ingredients at 4-7°C (Figure 2). One liter each of skim phase and sweet cream buttermilk were vat pasteurized at 68.3°C for 30 min and then cooled with ice to 12°C and placed in the cooler at 4-7°C. These dairy ingredients subsequently will be called “pasteurized” dairy ingredients.

Light Oxidation of Pasteurized and Unpasteurized Dairy Ingredients. Five hundred milliliters of pasteurized and unpasteurized skim and liquid buttermilk (samples that were only pasteurized in the cream phases) samples were placed in 500 mL clear, glass bottles in a Tonka refrigeration unit (Tonka, Hopkins, MN), under a row of fluorescent Econ-o-watt lights (Econ –O-Watt 1100-1300 lux) (VanAardt et al., 2000). The bottles (n=8) were placed in a randomized order, 12 inches from the light in the refrigeration unit to ensure even radiation of the bottles for 16 hours. Headspace was limited to 2cm from the top of the bottle to minimize atmospheric O₂. Half of the treatments were to be light

exposed (n=4) and the other half (n=4) were not “light exposed”. The samples that were not “light exposed” were stored under the same conditions in the same refrigeration unit, but the bottles were completely covered in aluminum foil.

Figure 2. Flow chart of pasteurization and light oxidation of dairy ingredients.



Composition of Dairy Ingredients. Fat, protein, moisture, and ash of fluid buttermilk and skim phase were determined. The protein content was analyzed using a dye binding method (DC Bio Rad assay, Bio Rad Laboratories, Hercules, CA). Fat content was calculated using the Modified Babcock Procedure in Standard Methods for the

Examination of Dairy Products (Marshall, 1993). Moisture and ash were determined by standard methods (Marshall, 1993).

Analysis of Volatile Flavor Components using Solid Phase Microextraction-Gas

Chromatography/Flame Ionization Detection (SPME-GC/FID). Extraction of volatile flavor components was completed using solid phase microextraction (SPME). The milk component sample (7.5 mL) with 5 μ L of internal standard solution (1ppm 4-methyl-2-pentanone – 1 μ L IS + 1000mL of distilled water), 2 g of sodium chloride (Fisher Scientific, Cincinnati, OH) and a stirring bar were placed in a 22 mL amber glass gas chromatography vial with a black vitton septum (Supelco, Bellefonte, PA). SPME was performed with a 65 μ m polydimethylsiloxane -divinylbenzene (PDMS-DVB) fiber mounted in SPME manual holder assembly. The sample vial was placed in a 45-50° C water bath and stirred at high speed. After allowing 2 min for the sample to equilibrate to 45-50°C, the septum piercing needle of the SPME device was inserted through the vial septum, and the plunger on the apparatus pushed down into the headspace to expose the fiber for 15 min. Gas chromatography/flame ionization detection (FID) was used to analyze and identify volatile components in the products (Hewlett-Packard, model 5890 A, Avondale, PA). The analytical column was a 25m x .32 mm, 1.05 μ m, HP-5 capillary column (Supelco, Bellefonte, PA). The column temperature was initially set at 45°C, held for .5 min, heated to 180°C at a rate of 9°C/min, held 5 min, and heated to 240°C at a rate of 18°C/min. Helium was used as the carrier gas at a flow rate of 1.0 mL/min; the injector temperature was 280°C and the FID was 300°C. Two replicates were performed on the analysis of the eight treatments. Milk samples were spiked at 50, 100, 200, and 400 ppb concentrations with acetaldehyde, 2-butanone, 2-pentanone, methyl sulfide,

hexanal, and diacetyl and the standard curves were used to determine the concentrations in the milk samples with 4-methyl-2-pentanone as the internal standard (Fisher Scientific, Cincinnati, OH) (McNair, 1998).

Part II. Improving the Sensory Characteristics of Skimmed Milk by Adding Sweet Buttermilk

Nonfat milk beverages were formulated by adding either cream or fluid sweet cream buttermilk to the skimmed ingredient in appropriate proportions to attain a .3% milkfat composition. The liquid buttermilk beverages were 75% liquid buttermilk and 25% skimmed milk. The skim beverage was 99% skimmed milk and 1% cream. The fat level was verified using the Modified Babcock Procedure (Marshall, 1993). A Braun hand mixer was used to disperse the ingredients to achieve uniformity. The formulations then were homogenized, pasteurized and refrigerated as described previously. Fat, protein, ash, and moisture were measured for each of the samples as described previously.

Light Oxidation of Formulations. Each beverage formulation (1000mL), in clear glass bottles (Pyrex), were placed in the Tonka refrigeration unit (Tonka, Hopkins, MN), under a row of fluorescent Econ-o-watt lights (Econ -O-Watt 1100-1300 lux) (VanAardt et al., 2000). Four bottles per formulation were placed in a randomized order in the refrigeration unit to ensure even radiation of the bottles for 16 h at 12 inches from the light. Headspace was limited to 2cm from the top of the bottle to minimize atmospheric O₂. Half of the treatments were light exposed (n=2) and the other half (n=2) were not “light exposed”. The samples that were not “light exposed” were stored under the same

conditions in the same refrigeration unit, but the bottles were completely covered in aluminum foil.

Sensory Analysis. A triangle test was used to evaluate overall difference between the light oxidized and non-light oxidized milk formulations. Specific comparisons were:

- 1) Pasteurized light oxidized liquid buttermilk beverage formulation→
Pasteurized light oxidized skim milk beverage formulation
- 2) Pasteurized liquid buttermilk beverage formulation→ Pasteurized skim
beverage formulation(control)

Eighteen untrained panelists between the ages of 15-55 from the faculty, staff and students in the Food Science and Technology Department at Virginia Tech received two sample sets in the sensory laboratory under fluorescent lights in individual booths in the Food Science and Technology Building at Virginia Tech (Meilgaard et al., 1999). The subjects received a tray with a score sheet, a napkin, a pencil, a cup of room temperature water, an empty cup to expectorate into, and three cold 15 mL samples (5-8°C) served in 2 oz. plastic soufflé cups. Each subject received two sets of three coded and randomized samples, and were asked to choose the different sample. Each set included two different sample combinations.

A hedonic test was used to evaluate preference. Ten panelists selected based on self identification as nonfat milk drinkers, received one set of four samples: oxidized liquid buttermilk beverage, unoxidized liquid buttermilk beverage, oxidized skim milk beverage, and unoxidized skim milk beverage, in the sensory laboratory in the Food Science and Technology Building at Virginia Tech (Meilgaard et al., 1999). The subjects received a tray with a score sheet, a napkin, a pencil, a cup of room temperature water, an

empty cup to expectorate into, and four cold (5-8°C) 15 mL samples in a 2oz. plastic soufflé cup. Each subject received four coded and randomized samples, and were asked to evaluate the samples on a scale from 1-9 with 9 being “likes extremely” and 1 being “dislikes extremely”. The hedonic test was replicated twice.

Statistical Design. The statistical design was a balanced complete block design. There were three replications on all analyses. The alpha level for all of the analytical analysis was .05 and Analysis of Variance (ANOVA) was performed on all the data using SAS Statistical Package (SAS, Cary, NC). The sensory tests were analyzed at an alpha level of .05, using the critical number of correct responses for the triangle test and ANOVA for the Hedonic test (Meilgaard et al., 1999).

RESULTS AND DISCUSSION

Part I. Evaluation of Flavor Components of Dairy Ingredients

Gross Composition and Color of Components

Table 2 reports fat, protein, moisture and ash concentration using standard methods (Marshall, 1993). There was no significant difference ($p > .05$) between the treatments in the moisture, ash, or protein. The liquid buttermilk ingredient contained higher fat concentrations ($p < .05$) than the skimmed milk components (0 mg/100mL skimmed milk). During the churning process the MFGM emulsion breaks and MFGM is released into the sweet cream buttermilk phase (Hui, 1993). Buttermilk contains lipid components associated with the MFGM such as phospholipids, cholesterol, and mono- and diglycerides that are not found in high concentrations in skimmed milk.

Table 2. Gross composition of pasteurized or unpasteurized components with or without light exposure.

Treatments ¹	Fat ² (mg/1000ml) x ± s.d.	Protein (mg/1000ml) x ± s.d.	Moisture (%) x ± s.d.	Ash (%) x ± s.d.
Spu	0	3.0 ± .7	91.2 ± .2	.04 ± .003
Suu	0	2.3 ± .7	91.6 ± .4	.03 ± .003
Spo	0	2.8 ± .8	90.3 ± .3	.04 ± .003
Suo	0	2.4 ± .8	91.9 ± .6	.04 ± .003
Lpu	.60 ± .01	2.9 ± .4	90.9 ± .1	.03 ± .003
Luu	.55 ± .05	2.8 ± .6	90.8 ± .4	.03 ± .003
Lpo	.57 ± .08	2.5 ± .7	90.1 ± .7	.04 ± .002
Luo	.48 ± .1	2.0 ± .6	90.6 ± .3	.03 ± .002

¹L = liquid buttermilk; S = skimmed milk; po = pasteurized oxidized; pu = pasteurized unoxidized; uu= unpasteurized unoxidized; uo= unpasteurized oxidized

²P<.05 x ± s.d. calculated for n=3

The color of nonfat milk is not as white as lowfat or whole milk, and is therefore, not as pleasing to the consumer (Phillips et al., 1995a). This difference in white appearance is due to compositional differences, with fat playing an important role. There

were minor differences in color attributes between skimmed milk and liquid buttermilk ingredients (Table 3).

Table 3. L*, a*, b* values¹ for skimmed milk and liquid buttermilk.

Treatments ²	L *	a*	b* ³
	(x ± s.d.)	(x ± s.d.)	(x ± s.d.)
Spu	73.9 ± 3.5	-56.9 ± 1.3	30.0 ± 1.4
Suu	70.9 ± 8.7	-53.3 ± 4.9	28.3 ± 2.5
Spo	75.7 ± 2.1	-56.0 ± 1.7	27.8 ± 1.1
Suo	73.1 ± 5.0	-55.3 ± 3.1	27.6 ± 1.4
Lpu	76.9 ± 1.2	-56.2 ± 1.5	31.6 ± 1.1
Luu	77.1 ± .75	-56.2 ± 1.4	29.7 ± .77
Lpo	78.1 ± .61	-57.0 ± .6	30.0 ± .39
Luo	77.4 ± .54	-56.6 ± .9	31.0 ± 1.5

¹L* 100 = white; 0 = black; a* 60 = red ; -60 = green; b* 60 = blue ; -60 = yellow;

²L = liquid buttermilk S = Skimmed milk; po= pasteurized oxidized; pu= pasteurized unoxidized; uu= unpasteurized unoxidized; uo= unpasteurized oxidized

³P<.05 x ± s.d. calculated for n=3

There was no significant difference ($p > .05$) in the L* or a* values among the treatments, but there was a significant difference ($p < .05$) in the b* value. The b* value represents the reflectance and absorbance of wavelengths giving the colors ranging from blue to yellow. Light scattering is caused by particles of larger molecular dimensions whose refractive index differs from the surrounding medium (Walstra and Jenness, 1984). In milk, these molecules are casein micelles and fat globules. The b* values are slightly lower for skimmed milk than the liquid buttermilk ingredient because casein micelles scatter the shorter wavelengths of visible light giving skimmed milk its blue hue (Hui, 1993). The liquid buttermilk treatments have higher b* values due to the higher fat content, which is shown in the proximate analysis data (Table 2).

Phillips et al. (1995a) reported that higher fat content of milk caused a corresponding increase in milk viscosity, which also affected milk color. As the milk viscosity increased, the milk samples were whiter, less blue (higher b* value) and less green (higher a* value) (Phillips et al., 1995a). This analytical difference is consistent

with preliminary data collected on milk beverages (.5% milkfat) formulated with liquid buttermilk, cream or dry buttermilk using a hedonic scale and a “just right” scale. The preliminary data suggested that the milk beverage formulated with liquid buttermilk had more yellow coloring than a milk beverage made with cream when evaluated using a “just right” test.

Volatile Flavor Analysis of Ingredients using Solid Phase Microextraction/Flame Ionization Detection. Liquid buttermilk, with a higher fat content than skimmed milk, has the potential of contributing more flavor volatiles to dairy beverages. The concentrations of the aldehydes were calculated using the internal standard method. Figure 3 displays the coefficient of error of the linear regression for the standard concentrations, which were used to calculate the concentrations of the volatiles in the skimmed milk and liquid buttermilk ingredients. These values were very close to 1.00 demonstrating the accuracy and precision of the SPME technique that was used for the extraction of the volatiles and the subsequent analysis by gas chromatography and flame ionization detection.

Acetaldehyde, 2-butanone, 2-pentanone, methyl sulfide, diacetyl, and hexanal are characteristic flavor compounds for pasteurized and light oxidized milk (Scanlan et al., 1968; Rerkrai et al., 1987). 2-Butanone, 2-pentanone and acetaldehyde are commonly found in light oxidized milk at higher concentrations than in fresh milk. Diacetyl, methyl sulfide and acetaldehyde are found in light oxidized milk but are found also in high concentrations in pasteurized milk contributing to the cooked/burnt protein flavor (Rerkrai et al., 1987).

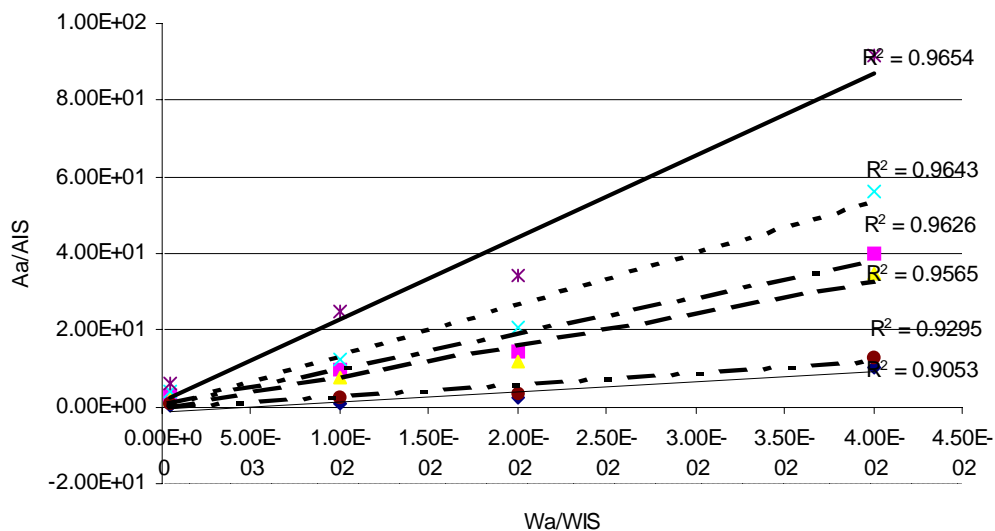


Figure 3. Milk samples were spiked with 50, 100, 200, and 400 μm samples to make 50, 100, 200, and 400 ppb concentrations of hexanal (.965), 2-pentanone (.964), methyl sulfide (.963), 2-butanone (.959), diacetyl (.930), and acetaldehyde (.905). The R^2 values were calculated to determine accuracy and precision of technique. The graph is plotted Area of analyte/Area Internal Standard vs. Weight of analyte/Weight of Internal Standard.

The concentrations of these aldehydes and ketones vary with the type of treatment and the component that is evaluated. The liquid buttermilk component has more flavor volatiles than the skim component due to the increased fat content of liquid buttermilk (Figure 4 and Figure 5).

Table 4 describes the concentration of volatile compounds in the dairy ingredients. The concentrations were determined using the internal standard method. The concentrations for acetaldehyde were consistent with concentrations in skim milk reported by Bassette (1976) and VanAardt et al. (2001) at between 14.2 ppb – 22.5 ppb.

Pasteurized unoxidized skim milk, which was considered the control, had measurable levels of 2-butanone, acetaldehyde, and diacetyl. Pasteurization of skimmed milk increased concentration of 2-pentanone and methyl sulfide to measurable levels. However only 2-butanone and acetaldehyde were detectable in oxidized skimmed milk. All liquid buttermilk ingredient treatments had measurable concentrations of 2-butanone, 2-pentanone, and acetaldehyde. Pasteurization of unoxidized liquid buttermilk increased the concentration of diacetyl and hexanal to measurable levels whereas oxidized buttermilk, both pasteurized and unpasteurized, had measurable levels of hexanal and methyl sulfide.

The light oxidized treatments should have had a significantly higher concentration of 2-butanone than the unoxidized treatments for both the buttermilk component and the skim component. 2-Butanone, acetaldehyde and 2-pentanone all contribute to an oxidized flavor. Oxidized aldehydes such as methyl sulfide also result from the sulfur compounds derived from the enzymatic breakdown of carbohydrates and protein compounds.

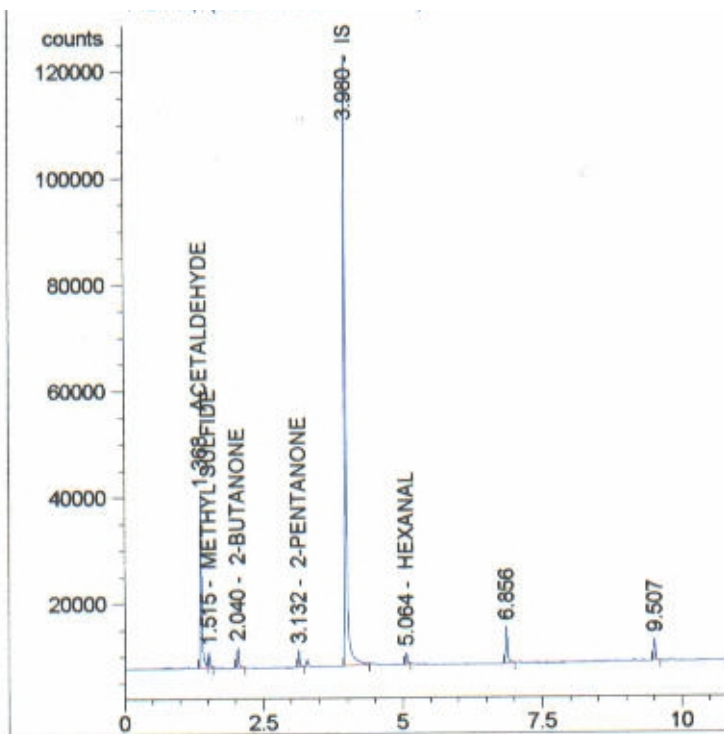


Figure 4. Gas chromatogram of pasteurized and oxidized liquid buttermilk with 4-methyl-2-pentanone as the internal standard. Acetaldehyde, dimethyl sulfide, 2-pentanone, and hexanal were identified using retention times of standards.

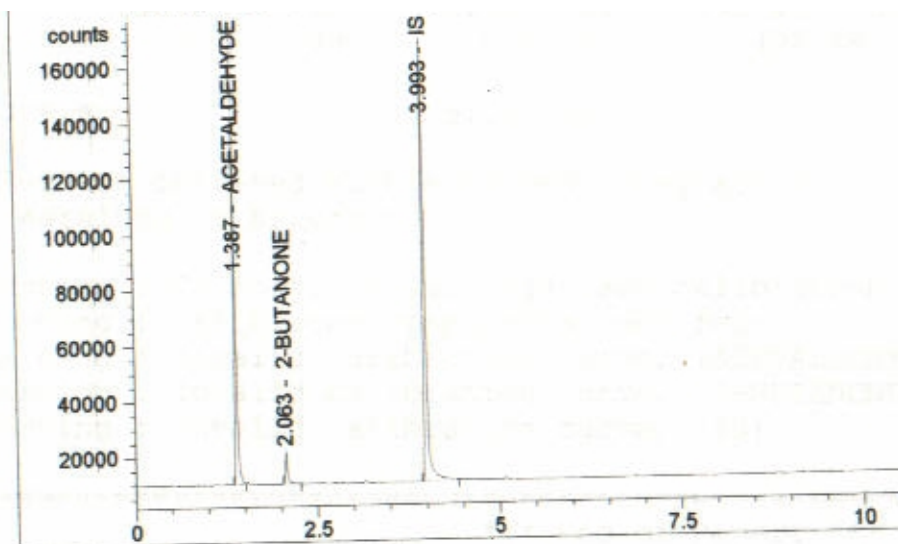


Figure 5. Gas chromatogram of unpasteurized and oxidized skim with 4-methyl-2-pentanone as the internal standard. Acetaldehyde and 2-butanone were identified using retention times of standards.

Table 4. Volatile flavor concentrations (ppb) in skimmed milk and liquid buttermilk components.

Treatment ¹	2-butanone (ppb) x ± s.d.	2-pentanone (ppb) x ± s.d.	Acetaldehyde (ppb) x ± s.d.	Diacetyl (ppb) x ± s.d.	Hexanal ² (ppb) x ± s.d.	Methyl Sulfide ² (ppb) x ± s.d.
Spu	.65 ± .49	.20 ± .31	14.2 ± 8.3	.14 ± .35	.19 ± .3 ^a	n.d.* ^a
Suu	.76 ± .57	n.d.*	21.2 ± 19.2	.33 ± .51	n.d.* ^b	n.d.* ^a
Spo	.68 ± .53	n.d.*	17.2 ± 15.4	n.d.*	n.d.* ^b	n.d.* ^a
Suo	.75 ± .54	n.d.*	19.8 ± 15.6*	n.d.*	n.d.* ^b	n.d.* ^a
Lpu	.82 ± .57	.24 ± .19	17.1 ± 9.5	.18 ± .44	.53 ± .2 ^a	n.d.* ^a
Luu	.89 ± .70	.19 ± .20	19.7 ± 13.3	n.d.*	n.d.* ^b	n.d.* ^a
Lpo	.81 ± .57	.26 ± .69	20.4 ± 15.0	n.d.*	n.d.* ^b	.18 ± .27 ^b
Luo	1.0 ± .71	.26 ± .74	22.5 ± 18.9	n.d.*	n.d.* ^b	.21 ± .34 ^b

¹L = liquid buttermilk; S = skimmed milk; po = pasteurized oxidized; pu = pasteurized unoxidized; uu = unpasteurized unoxidized; uo = unpasteurized oxidized;

* nd = not detected

²P<.05 x ± s.d. calculated for n=3; and b represent significant difference within columns

Hexanal, diacetyl and methyl sulfide are aldehydes and ketones that have been shown to be affected by various heat treatments (Rerkrai et al., 1987). In this study, hexanal and diacetyl were found in pasteurized and unoxidized treatments, and methyl sulfide was detected only in oxidized liquid buttermilk. Hexanal was found at a higher concentration in liquid buttermilk than in skimmed milk. Heat treatments have been shown to denature proteins and cause chemicals to be derived that give off-flavors to milk. Other flavor compounds, including alkanes, lactones, esters, sulfur compounds, nitrogen compounds, aliphatic compounds and other aromatic hydrocarbons that occur naturally in the milk, are caused by light oxidation or pasteurization, or a combination of both during storage (Whaemi et al., 1988).

Proteins also have an impact on flavor of the milk. Enzymes such as proteases and lipases affect the flavor and stability of milk (Varnam and Sutherland, 1994). When milk is pasteurized, different types of heating flavor can occur. Low-pasteurized milk (73°C for 10s) is almost free of heating flavors caused by proteins, but high pasteurized milk and cream (83°C for 10s) have a cooked flavor profile caused by hydrogen sulfide

compounds that are formed through thermal conversion of milk proteins (Walstra and Jenness, 1984). Oxidoreductases affect the flavor especially in the lipid fraction, which is critical to the shelf stability of whole milks. The average protein composition of sweet buttermilk is 28.35mg/g buttermilk (Elling et al., 1996). Proteins in skimmed milk which include the caseins and whey proteins, β -lactoglobulin and α -lactalbumin, constitute about 75% of the total protein in milk (Corredig and Dalgeish, 1998). While there was no difference in total protein composition of liquid buttermilk and skimmed milk, the milk fat globule membrane fragments in the liquid buttermilk provides more enzyme and different proteins than found in skimmed milk (Elling et al., 1996)

Part II. Improving the Sensory Characteristics of Nonfat Milk by Adding Sweet Buttermilk

Preliminary Data on Dairy Ingredients for Nonfat Milk Beverages. There are many underutilized dairy ingredients, such as sweet cream buttermilk, nonfat dry milk, dry buttermilk and butter-derived aqueous phase, which have a high concentration of flavor compounds that can be used to enhance the flavor of nonfat milks without adding as much fat as cream. Preliminary data was collected to determine which dairy ingredients would be evaluated in formulated milk beverages. Several of these underutilized dairy ingredients were eliminated due to lack of consistency of the ingredients and the formulated beverages. There were variations in total solids, proteins, beverage appearance, and large differences in the flavor when proximate and sensory analyses were done on nonfat beverages formulated with nonfat dry milk and dry buttermilk (Table 5). Preliminary data was collected using butter derived aqueous phase, but the visual appearance of the final product was poor and would not allow for consistent sensory data due to the appearance of yellow butter flakes in the final beverage.

Table 5. Preliminary data for dairy beverages using liquid buttermilk, nonfat dry milk, dry buttermilk and skim milk.

Treatment	Fat (%) x ± s.d.	Protein (%) x ± s.d.	Moisture (%) x ± s.d.	Ash (%) x ± s.d.
Skim	0	1.45 ± .49	92.1 ± .09	.03 ± .003
Nonfat dry milk	.25 ± .07	1.71 ± .56	83.3 ± .06	.03 ± .003
Liquid buttermilk	.3 ± 0	1.86 ± .49	91.3 ± .14	.03 ± .003
Dry buttermilk	.31 ± .05	2.76 ± .23	86.3 ± 1.1	.03 ± .003

x ± s.d. calculated from n=3

Composition and Color of Milk Beverages Using Skimmed Milk or Liquid Buttermilk

Components. Nonfat milk beverages formulated to .3% milkfat using either liquid buttermilk or cream with skimmed milk were not different ($p > .05$) in gross composition (Table 6). Standardized composition was important so that any flavor or color differences that might be noted could be attributed to the ingredient and not variations in gross composition.

Table 6. Proximate analysis of fat, protein, moisture and ash of the nonfat (.3%) milk formulations using liquid buttermilk or skimmed milk components.

Treatment ¹	Fat (%) x ± s.d.	Protein (%) x ± s.d.	Moisture (%) x ± s.d.	Ash (%) x ± s.d.
Su	.3	3.7 ± 1.2	90.6 ± .5	.038 ± .002
So	.3	3.6 ± 1.3	91.1 ± .3	.025 ± .002
Lu	.3	3.4 ± 1.3	90.7 ± .2	.025 ± .002
Lo	.3	3.4 ± 1.3	90.7 ± .3	.03 ± .003

¹L = Liquid buttermilk formulations; S = Skim milk formulations; o = oxidized; u = unoxidized

² $P < .05$ x ± s.d. calculated for n = 2

There was a significant difference ($p < .05$) in the L* values of the formulations but no significant differences were found in the a* or b* values among the formulations (Table 7). This difference exists between the unoxidized and oxidized skim treatments of the beverages formulated with skim and cream. After oxidation, there are peroxides and carbonyls in milk, which can enhance milks opaque appearance, and thereby increasing the L value. Carbonyl compounds and peroxides are shown to absorb at long

wavelengths, which causes the medium that they are in to appear more opaque (Hui, 1993).

Table 7. Color values (L^* , a^* , and b^*)¹ for pasteurized nonfat milk beverages formulated with liquid buttermilk or cream with skimmed milk with or without light exposure.

Treatment ²	L^* x ± s.d.	a^* x ± s.d.	b^* ³ x ± s.d
Su	77.9 ± .22	-58.0 ± .10	30.1 ± .36
So	78.8 ± .26	-58.5 ± .26	29.5 ± .09
Lu	78.0 ± .22	-57.4 ± .28	30.1 ± .67
Lo	78.0 ± .32	-57.8 ± .19	30.2 ± .56

¹ L^* 100 = white ; 0 = black; a^* 60 = red ; -60 = green; b^* 60 = blue ; -60 = yellow

²L = Liquid buttermilk formulations; S = Skim milk formulation; o =oxidized; u = unoxidized

³ $P < .05$ x ± s.d. calculated for n = 2

Sensory Results for Formulations. Two sensory evaluation methods, a hedonic test and a triangle test, were performed on the milk formulations. Both of these tests were evaluated in duplicate. The hedonic test is an overall acceptance test that uses a scale to evaluate different treatments in terms of “dislikes extremely” to “likes extremely” with scores ranging from 1-9. There was no difference in the acceptability of the four treatments with means within the neither likes nor dislikes (x=5) to likes slightly (x=6) range.

The triangle test was used to determine overall difference between the unoxidized milk beverages formulated with buttermilk or cream and skimmed milk and between the oxidized milk beverage formulated with buttermilk or cream and skimmed milk. The test determined that there was no significant differences overall between any of the treatments at $p > .05$ and $\beta > .10$ (Table T7 and T8) (Meilgaard et al., 1999). These results agree with the analytical results. The concentrations of the volatiles measured in the components were low, possibly below sensory thresholds. Van Aardt et al. (2001) found the threshold for acetaldehyde in nonfat milk was 3939 ppb and we evaluated the acetaldehyde

concentration to be at 22.5 +/- 18.9 at its highest concentration in liquid buttermilk unpasteurized and oxidized component. The panelists could not have determined the difference between the treatments because the change in the concentrations of the treatments were undetectable at such low concentrations.

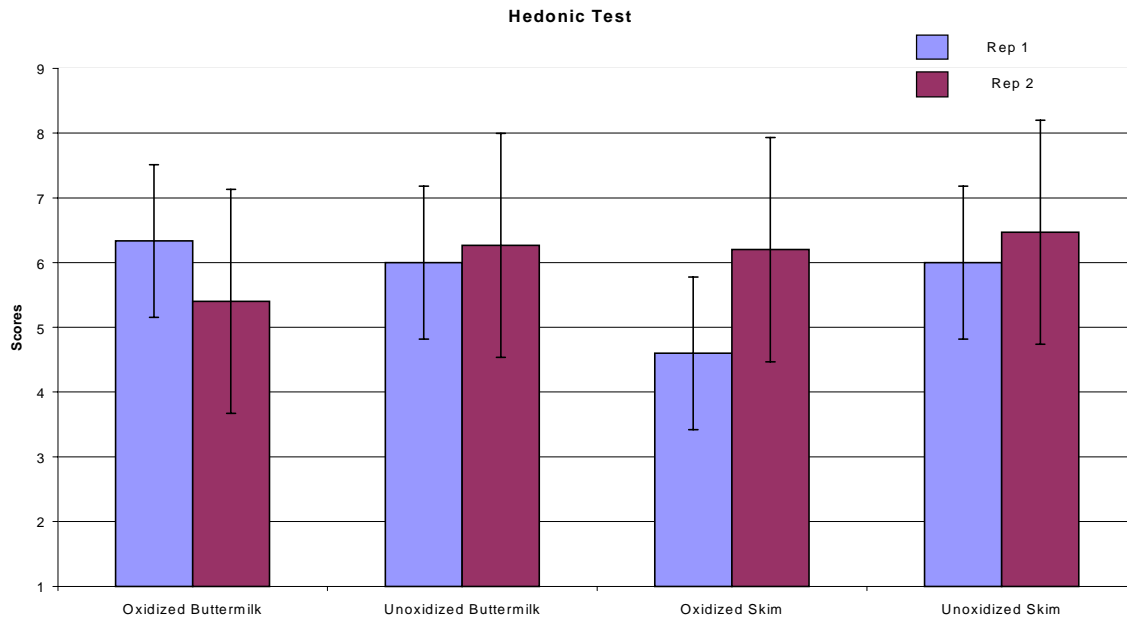


Figure 6. The hedonic scores for the oxidized and unoxidized milk beverages formulated with 75% liquid buttermilk and 25% skimmed milk (Buttermilk), and hedonic scores for the oxidized and unoxidized milk beverages formulated with 99% skimmed milk and 1% cream (Skim) for 2 replications were not significantly different.

CONCLUSIONS

Liquid sweet buttermilk used at relatively high percentages, 75%, in the formulation of a nonfat dairy beverage, does not contribute enough volatile compounds to improve the flavor of the beverage, as compared to the traditional nonfat milk formulated with cream and skim milk.

Changes in volatile chemistry due to oxidation of dairy ingredients were minimal, suggesting that the liquid buttermilk ingredient may be used in a beverage formulation without detrimental quality effects.

REFERENCES

- Bassette, R. 1976. Effects of light on concentrations of some volatile materials in milk. *J. Milk Food Technol.* 39:10-12.
- Bassette, R., Fung, D.Y.C., and Roberts, H. 1983. Effect of pasteurization temperature on susceptibility of milk to light-induced flavor. *J. Food Prot.* 46:416-419.
- Cadwallader, K. and Howard C.L. Analysis of aroma active components of light activated milk. In *Flavor Analysis: Development in Isolation and Characterization*, M. Morrello and C. Mussinan, Eds. ACS Symposium Series N. 705, American Chemical Society, Washington, DC, 1998 pp 343-358.
- Corredig, M. and Dalgleish, D. 1998. Buttermilk properties in emulsions with soybean oil as affected by fat globule membrane derived proteins. *J. Food Sci.* 63(3):476-480.
- Elling, J.L., Duncan, S.E., Keenan, T.W., Eigel, W.N., and Boling, J. 1996. Composition and microscopy of reformulated creams from reduced cholesterol butteroil. *J. Food Sci.* 61(1):48-52.
- Hui, Y.H. 1993. *Dairy Science and Technology Handbook*. VCH Publishers, Inc New York, NY.
- Marshall, R.T. 1993. *Standard Methods for the Analysis of Dairy Products*. American Public Health Association, 16th Ed, Washington, D.C, Port City Press, Baltimore, MD.
- Marsili, R.T. 1999. Comparison of solid phase microextraction and dynamic headspace Methods for the gas chromatographic mass spectrometric analysis of light induced lipid oxidation products in milk. *J. Chromat. Sci.* 37:17-23.
- McNair, H. 1998. *Gas Chromatography*. Department of Chemistry. Virginia Polytechnic Institute and State University. Blacksburg, VA.
- Meilgaard, M., Civille, G., Thomas, B. 1999. *Sensory Evaluation Techniques*. 3rd ed. CRC Publishers, Boca Raton, FL.
- Newstead, D. F. 1999. Sweet-cream buttermilk powders: Key functional ingredients for recombined milk products. In *Recombined Milk and Milk Products*. pp. 55-60, IDF Special Issue 9902. International Dairy Federation, Brussels, Belgium.
- Norman, G.H. 1955. Dried buttermilk improves palatability of reconstituted milk. *Milk Products Journal*. 46:38-39.

- Phillips, L. and Barbano, D. 1997. The influence of fat substitutes based on protein and titanium dioxide on the sensory properties of lowfat milks. *J. Dairy Sci.* 80:2726-2731.
- Phillips, L., McGiff, M.L., Barbano, D., and Lawless, H. 1995a. Influence of nonfat dry milk on the sensory properties of viscosity and color of lowfat milks. *J. Dairy Sci.* 78: 2113-2118.
- Rerkrai, S., Jeon, I., and Bassette, R. 1987. Effect of various direct ultra high temperature heat treatments on flavor of commercially prepared milks. *J. Dairy Sci.* 70:2046-2054.
- Scanlan, R.A., Lindsay, R.C., Libbey, L.M., and Day, E.A. 1968. Heat induced volatile compounds in milk. *J. Dairy Sci.* 51:1001-1006.
- van Aardt, M., Duncan, S.E., Bourne, D, Marcy, J.E., Long, T.E., Hackney, C.R. and Heisey, C. 2001. Flavor threshold for acetaldehyde in milk, chocolate milk and spring water using solid phase microextraction gas chromatography for quantification. *J. Ag. Food Chem.* 49(3):1377-1381.
- Varnam and Sutherland. 1994. *Milk and Milk Products: Technology, Chemistry, and Microbiology*, Chapman and Hall, London.
- Walstra, P. and Jenness, R. 1984. *Dairy Chemistry and Physics*, Wiley and Sons, New York, NY.
- Whaemei, J., Bassette, R., and Crang, R. 1988. Effects of light and copper on volatile aldehydes of milk and milk fractions. *J. Dairy Sci.* 71:2366-2372.

APPENDIX A.
SENSORY

TRIANGLE TEST

Subject No. _____

Instructions

Taste the samples on the tray from left to right. Two samples are identical, one is different. Select the odd/different sample and indicate by circling the code number of the odd sample

Sample Numbers

_____ _____ _____
_____ _____ _____

Thank You!

Hedonic Scorecard

Location: _____

Judge Number: _____

Product: _____

Please taste sample _____, sample _____, sample _____ and then sample _____.
Indicate how much you like each sample checking the term that best describes your toward that sample.

	Sample	Sample	Sample	Sample
Like extremely	_____	_____	_____	_____
Like very much	_____	_____	_____	_____
Like moderately	_____	_____	_____	_____
Like slightly	_____	_____	_____	_____
Neither like nor dislike	_____	_____	_____	_____
Dislike slightly	_____	_____	_____	_____
Dislike moderately	_____	_____	_____	_____
Dislike very much	_____	_____	_____	_____
Dislike extremely	_____	_____	_____	_____



HUMAN SUBJECTS FORMS FOR SENSORY EVALUATION

PROTOCOL FOR PROJECTS OF SENSORY EVALUATION

Definition: Sensory evaluation is the evaluation of food or other substances by the senses including taste, touch, smell, sight and hearing.

Check all that apply:

1. The procedure for sensory evaluation in this project involves:
 - Tasting in the mouth (includes tests where the panelist is instructed to spit it out)
 - Substances applied to the skin
 - Substances smelled for odor components
 - Substances evaluated by sound when chewed
 - Substances evaluated by visual senses

2. The product/s to be evaluated are:
 - Made entirely of ingredients approved by FDA for consumption or application under approved conditions of processing
 - Made of ingredients approved by FDA but not approved for the use in the project (e.g. heating of aspartame, fat substitutes approved only as an emulsifier).
 - Made partially or entirely of experimental ingredients pending FDA approval.
 - Made partially or entirely of experimental ingredients not approved for human consumption or topical use
 - Made from materials from or altered by biotechnology

3. The processing or preparation of the product is:
 - By usual approved good manufacturing or preparation practices for that food or topical product.
 - By experimental procedures including non-good manufacturing practices. Briefly describe the procedures.

4. The packaging of the product includes:
 - Processing or storage in FDA-approved packaging materials.
 - Processing or storage in packaging materials not approved by FDA.

5. Describe the storage protocols for the product that are necessary to maintain the product in safe condition.

Milk will be stored in a sealed glass container at 4-7°C until sensory testing.

6. If microbiological cultures are a part of the food processing or preparation procedure, describe what cultures will be used, if they will be active on consumption, and give evidence that these cultures are known to be safe for human consumption.

No microbiological cultures used

7. Allergies

Yes Are any ingredients to be used potentially allergenic as consumed or by topical application? If yes, describe. Have panelists been made aware of these ingredients?

Lactose Intolerance and milk proteins

When you have completed this form, indicate the risk level to the panelists of this project. Complete the appropriate form; for "not at risk", the Certificate of Exemption form; for "at minimal risk", the Request for Approval form.

**VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY
INFORMED CONSENT FOR PARTICIPATION IN SENSORY EVALUATION**

Title of Project: *The Sensory and Analytical Analysis of Nonfat Sweet Cream Buttermilk Formulations*

Principal Investigator: *Dr. Susan E. Duncan, Ph.D., R.D., Associate Professor*

I. THE PURPOSE OF THIS PROJECT

You are invited to participate on a sensory evaluation panel about milk.

The purpose of the project is to evaluate the flavor, color, and viscosity of a variety of milk formulations using liquid sweet cream buttermilk, dry sweet cream buttermilk, nonfat dry milk, aqueous phase, and nonfat liquid milk.

II. PROCEDURES

There will be 2 sessions over a period of 2 weeks involving about 15 minutes at each session. You will be presented with approximately 6 samples at each session. As a panelist, it is critical to the project that you attend each session. Should you find a sample unpalatable or offensive, you may choose to spit it out and continue to other samples.

Certain individuals are sensitive to some foods such as milk, eggs, wheat gluten, strawberries, chocolate, artificial sweeteners, etc. If you are aware of any food or drug allergies, list them in the following space.

III. BENEFITS/RISKS OF THE PROJECT

Your participation in the project will provide information that may be helpful to the determining a difference and a preference amongst different lowfat milk formulations. You may receive the results or summary of the panel when the project is completed. Some risk may be involved if you have an unknown food allergy.

IV. EXTENT OF ANONYMITY AND CONFIDENTIALITY

The results of your performance as a panelist will be kept strictly confidential. Individual panelists will be referred to by code for analyses and in any publication of the results.

V. COMPENSATION

Please accept a small piece of candy, as a token of our appreciation for your participation in this project.

VI. FREEDOM TO WITHDRAW

It is essential to sensory evaluation projects that you complete each session in so far as possible. However, there may be conditions preventing your completion of all sessions. If after reading and becoming familiar with the sensory project, you decide not to participate as a panelist, you may withdraw at any time without penalty.

VII. APPROVAL OF RESEARCH

This research project has been approved by the Institutional Review Board for projects involving human subjects at Virginia Polytechnic Institute and State University and by the human subjects review of the Department of Food Science and Technology.

VIII. SUBJECT'S RESPONSIBILITIES

I know of no reason I cannot participate in this study which will require sensory evaluation of milk and milk products.

Signature/Date

Please provide address and phone number so investigator may reach you in case of emergency or schedule changes.

Address: _____

Phone: _____

E-mail: _____

IX. SUBJECT'S PERMISSION (provide this page for human subject to keep)

I have read the information about the conditions of this sensory evaluation project and give my voluntary consent for participation in this project.

I know of no reason I cannot participate in this study, which will involve sensory analysis of milk or milk products.

Signature

Should I have any questions about this research or its conduct, I should contact:

Dr. Susan E. Duncan / (540) 231-8675
Investigator/Phone

Food Science and Technology / (540) 231-6806
Faculty/Phone

Dr. David Moore / (540) 231-4991
Chair, IRB/Phone for Research Division

OUTLINE FOR PROTOCOL TO ACCOMPANY IRB REQUEST

JUSTIFICATION OF PROJECT

The objective of this project is to enhance the flavor, viscosity, and appearance of nonfat milks as compared to nonfat products currently available. The project will evaluate the flavor, color and viscosity of an underutilized dairy product, sweet cream buttermilk, as an ingredient. A sensory panel will evaluate the nonfat formulations of fluid milk with buttermilk as an ingredient. This sensory data will be supported with physical property measurements and analytical flavor chemistry using the SPME-GC/MS.

PROCEDURES

Human subjects will be selected at random with regards to age, sex, etc.

Raw milk will be obtained from the Virginia Tech dairy farm and separated into cream and skim phase using a pilot plant separator (Elecrem separator, mode IG, 6400 rpm, Bonanza Industries, Inc., Calgary, Alberta). Cream and skim phase will be vat pasteurized at 68.3 °C. Cream will be heated to 13-14°C and incubated overnight. Sweet buttermilk will be made by mechanically churning the cream at 16.7°C. Milk samples will be formulated using nonfat dry milk and liquid nonfat milk, liquid sweet cream buttermilk, dry sweet cream buttermilk (Land O'Lakes, Arden Hills, MN) and liquid nonfat milk, and liquid nonfat milk. The samples will be stored at 4-7°C until time of analysis.

There will be only one sensory session per day of sensory analysis, which will take 15 minutes per session. Each human subject can be asked to participate in more than one sensory session, but is under no obligation. No training prior to sessions will be expected from the human subjects. The project will be replicated three times.

Another investigator of the project who will have contact with the human subjects is Jodi Powell (B.S in Chemistry).

Find attached an example of a questionnaire that will be used.

RISKS AND BENEFITS

Participation in this project will provide information that may be useful in developing uses of an underutilized dairy ingredient, enhancing sensory properties and nutritional value of nonfat milk. Each human subject may receive the results or summary of the panel when the project is completed. Some risk may be involved if you have an unknown food allergy.

CONFIDENTIALITY / ANONYMITY

The results of each panelist will be kept strictly confidential and will only be accessible to the investigators of this study. The individual panelists will be referred to by code for analysis and in any publication of the results.

VITA

Jodi Powell was born in Washington, D.C. to Mr. and Mrs. Adrian Powell. She attended Oxon Hill Senior High School in the Science and Technology program from 1990-1994. After graduation from high school she attended Delaware State University on a full USDA/1890 National Scholar Scholarship Award and the Delaware State University Presidential Scholar Scholarship Award from 1994-1998 where she attained her Bachelors of Science in Chemistry. She then entered Virginia Polytechnic Institute and State University in the Masters of Science Program in Food Science and Technology under Susan E. Duncan as her advisor. She has been accepted into a PhD program at Alabama A&M in Huntsville, AL in the Department of Food and Animal Science for a PhD in Food Science and Technology.