COMPARISON OF SENSORY CHARACTERISTICS, AND INSTRUMENTAL FLAVOR COMPOUNDS ANALYSIS OF MILK PRODUCED BY THREE PRODUCTION METHODS

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ABSTRACT

The objectives of this research were to carry out an analytical and sensory comparative studies on the flavor constituents of milk from three production systems: organic (O), pasture-feed based system (P), and conventional (C). The volatiles compounds were extracted from the milks with a DVB/CAR/PDMS SPME fiber and transferred into GC-MS for identification and quantification. Statistical analysis showed significant differences between C and P milk on hexanal, pentanal, octanal and nonanal content which were attributed to the degree of lipid oxidation between milks. An unidentified compound at retention time of 3 minutes was suggested as a discriminating compound for the three type of milks, and pentanal was pointed out as a possible discriminator compound for organic milk.

From the preference test, we concluded that panelists clearly differentiated organic milk from conventional and milk from pasture-fed cows for their overall flavor, liking, and mouthfeel, whereas distinction between conventional and milk from pasture-fed cows was only achieved for overall appearance. These results

were in agreement with a triangle test which showed that panelists clearly differentiated organic milk from the rest. Pentanal, as well as a common set of potent odorants that were only present in the organic milk chosen for the sensory study, were likely responsible for the ability of the panelists to distinguish the organic from the other two milks.

CHAPTER 1

INTRODUCTION

Milk has been part of the American diet since 1611, when the first cows were brought to Jamestown, Virginia (IDFA, 2005). The consumption of milk in the USA reached its peak in 1945 with nearly 41 gallons per person per year. Since then, the consumption of milk has decreased due to consumer's concerns about the high amount of cholesterol, saturated fatty acids, and calorie content found in milk. Furthermore, the popularity of soda among teenagers, and the proliferation of a more ethnically diverse population whose diet does not include milk, helped decrease milk consumption (Waves, 2003).

In 1993, the rBGH (recombinant bovine growth hormone) was approved for injection into cows with the aim of increasing milk yield. Public concerns with the rBGH have led to a boom of the organic milk market, despite the fact that there is no scientific consensus on the potential harmful effects of rBGH on human health. The widespread belief among organic milk consumers is that organic milk has superior qualities over conventional milk. However, organic milk superiority has not been scientifically proven (DuPuis, 2000). In fact, inconsistent findings have been found in comparing conventional versus organic farming systems, as well as in comparing organic versus conventional milk (Siderer et al., 2005). Thus, food researchers have commonly agreed

that more research needs to be done in that area in order to evaluate the differences between organic and conventional milk.

Along with the steady growth of the organic milk market, there has also been a rising demand for milk from cows that are allowed to pasture. Pasture-based systems share many similarities with organic based systems although they do not subscribe to the organic standards set by the NOP (National Organic Program) (Hale, 2006).

Sensory properties of milk, such as flavor, are extremely important to the dairy industry because they directly correlate product quality and consumer acceptance. Milk has a bland, yet characteristic flavor, which is very susceptible to develop off-flavors due to heat treatments, enzymatic and microbial activities, oxidation processes, transfer of substances from the feed, and transfer of substances from the environment. The flavor profile of milk is a complex mixture of several volatiles compounds that is affected by several factors such as type of cow's feed, percentage of milk fat, heat treatment, packaging, and storage time (Parliament et al., 2000). Traditionally, the characterization of milk's flavor profile was done by distillation methods, but the invention of SPME (Solid-phase microextraction) in 1989 by professor Pawliszyn provided flavor scientist with a more efficient, solventless, and fast sample preparation technique (Vas et al, 2004).

Since the beginning of its foundation, the dairy industry has used a system known as a Score Card to evaluate milk sensory properties. The Score Card system consists of the evaluation of potential defects of the milk by a highly

trained judge (Bodyfelt et al., 1998). However, this approach has been criticized for its failure to predict consumer acceptance, its lack of objectivity in quality judgments, and the complexity in assignment of quantitative scores (Chapman et al., 2001). As a result, the dairy industry is moving towards other types of sensory studies more suitable for the evaluation of the sensory attributes of milk (Lawless and Heymann, 1993).

No information could be found in the literature on the differences of flavor profile of organic milk, milk from pasture-fed cows and conventional milk. In addition, no information was found in the sensory evaluation of these three type of milks. Thus the objectives of this study were (1) to characterize the flavor profile of milk from three production systems that were labeled certified organic, pasture-fed based, and conventional by using SPME and Gas Chromatography/Mass Spectrometry (GC/MS) and (2) to evaluate milk's sensory attributes by using preference and discrimination tests.

CHAPTER 2

LITERATURE REVIEW

2.1 Flavor molecules from milk lipids

Flavor, the odor and taste sensation one receives in the process of chewing food, is the most important factor that influences the degree of liking for the food we eat. Volatile compounds present in the food compose its aroma, the strongest contributor to food's flavor (Reineccius, 2006).

Milk has a bland, yet characteristic flavor, which can be modified by heat treatment, enzymatic and microbial activity, oxidation process, transfer of substances from the feed, and transfer of substances from the environment (Parliament et al., 2000). Several studies have been carried out on the volatiles constituents of milk compiling a list of more than four hundred compounds (Moio et al., 1994). These compounds can be divided into five categories:

➢ Compounds derived from lactose or citrate as a product of lactic flora (*lactoccocci* bacteria), such as lactic acid, and acetaldehyde.

➢ Compounds produced as a result of reactions involving milk's fat such as hydroxy acids, lactones, aldehydes, and methyl ketones.

➢ Strecker aldehydes produced as a result of the breakdown of casein, other milk proteins, and amino acids.

➢ Sulfur compounds derived from cysteine and methionine.

➢ Compounds produced as a result of Maillard reactions, such as furanones, maltol, and 2-acetylfuran (Parliament et al., 2000).

Special emphasis will be given to compounds produced as a result of lipid reactions because milk fat is the origin of most of the flavors encountered in milk (Harper and Hall, 1976). Lipids undergo many reactions such as hydrolysis, autoxidation, ß-oxidation, dicarboxylation, dehydration, reduction and esterification producing several different classes of flavorful compounds, such as fatty acids, ketones, lactones, aldehydes, alcohols, hydrocarbons, and esters (Parliament et al., 2000).

1. Fatty acids. Fatty acids result from the hydrolysis of triglyglicerides. It has been found that the concentrations of acetic, butyric, hexanoic, octanoic, and decanoic acid increases when milk is heated above 100°C. Butyric and hexanoic acid are the major free fatty acids in skim milk and whole and skim milk powder, and they contribute to the chemical and rancid flavor of heated milk (Shibamoto, et al. 1980).

2. Esters. In raw milk, esters make up about half of the neutral volatile fraction, especially short-chain and medium-chain fatty acid ethyl esters of butyric, hexanoic, octanoic and decanoic acids, such as ethyl butanoate, ethyl hexanoate, and ethyl decanoate. In pasteurized and homogenized milk, esters are present in lower concentrations because heat treatments above 100°C cause their hydrolysis and subsequent formation of methyl ketones (Moio et al., 1993 a,b).

3.- Aldehydes. Most of the aldehydes found in milk are within the range of C_5-C_{11} such as pentanal, hexanal, heptanal, and nonanal (Moio et al., 1993a). Many of these aldehydes are formed by the oxidation of unsaturated fatty acids and their esters, although some are formed through secondary reactions. In the autoxidation process, oxygen (O_2) is added to a methylene group adjacent to a double bond, leading to the formation of hydroperoxides, which further decompose to straight-chain aldehydes. Autoxidation is catalyzed by trace metals, such as copper, and by enzymes (Forss, 1979). The most important precursors for the formation of aldehyde compounds are the polyunsaturated acids, including oleic, linoleic, linolenic and archidonic acid, due to their prevalence in milk products. Table 2-1 lists the possible origin of some aldehydes obtained from the oxidation of the previously mentioned acids (Shipe, 1980).

Aldehydes from non-lipid origin such as 3-methylbutanal, and benzaldehyde are also commonly found in pasteurized milk (Moio et al., 1994)**.**

4- Ketones. Ketones are mainly found in milk as a series of 2-methyl ketones with an odd number of carbon atoms (C_7, C_9, C_{11}, C_{12}), such as 2-pentanone, 2-heptanone, 2-nonanone, and 2-undecanone. Ketones arise from the oxidation of free fatty acids into ß-ketoacids and the decarboxylation of these into methyl ketones (Moio et al., 1993a). The concentration of methyl ketones depends on the degree of the heat treatment, and time of storage. Thus, methyl ketones are present in higher concentrations in UHT milk than in raw milk, and

in long-stored milk than in fresh milk (Badings et al., 1981). A proposed pathway

of the formation of methyl ketones is presented in Figure 2-1.

**Table 2-1. Possible origin of some aldehydes obtained from
the oxidation of oleic, linoleic, linolenic, and arachidonic acids.**

Fatty acid	Hydroxide position	Aldehyde obtained
Oleic	C11	Octanal
	C8	2-undecenal
	C9	2-decenal
	C10	Nonanal
Linoleic	C13	Hexanal
	C9	2,4- decadienal
	C11	2-octenal
Linolenic	C16	Propanal
	C14	2-pentenal
	C12	2,4-heptadienal
	C13	3-hexenal
	C11	2,5 octadienal
	C9	2,4,7- decatrienal
Arachidonic	C15	Hexanal
	C13	2-octenal
	C12	3-nonenal
	C11	2,4- decadienal
	C10	2,5- undecadienal
	C7	2,5,8-tridecatrienal

Shipe (1980).

Triglyceride

| Lipase

Fatty acids

| B-oxidation

Keto Acyl-CoA

| Thiohydrolase

Keto Acid
| Decarboxylase

Alkan-2-one (methyl ketone)
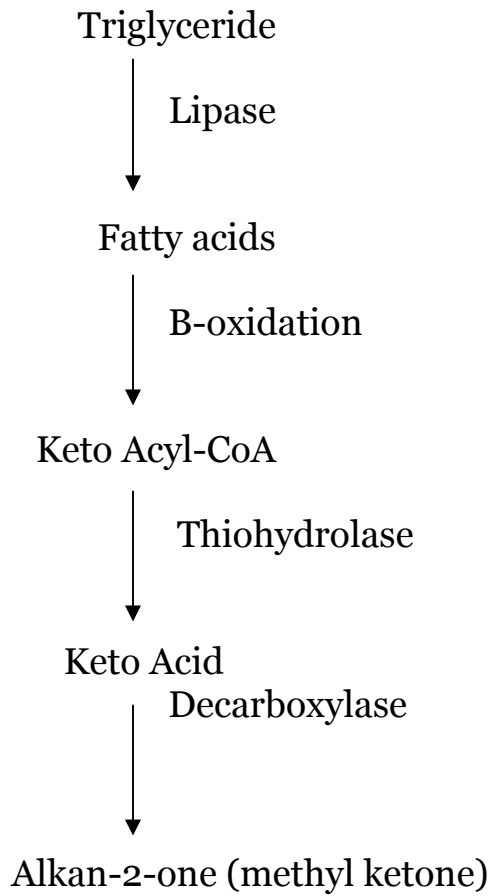
Figure 2-1.   Proposed formation pathway of methyl ketones .

Forss (1979).

5.- Alcohols. Several alcohols such as ethanol, 1-pentanol, 1-octen-3-ol, 1-octanol, dodecanol, tetradecanol, and pentanol have been encountered in pasteurized milk (Moio et al., 1993a; Toso et al., 2002). Primary alcohols are probably formed by reduction of the respective aldehyde (Moio et al., 1993a). The contribution of alcohols to the aroma of milk is almost negligible because they are relatively flavorless and also because they are present in low concentrations (Forss, 1972).

6.- Lactones. Very small amounts of lactones have been found in fresh unheated milk, which have been linked to raw milk and butter flavor. In fact, lactones are formed during heat treatments from the thermal breakdown of γ- and δ- hydroxyacids (Forss, 1972) (Fig 2-2). The following lactones have been identified in milk: δ-decalactone, γ -dodecalactone, 5-methyl-2 (5H) furanone, 2-butenoic, and acid- γ -lactone (Moio et al.,1993a).

$$R-\overset{5}{C}H-CH_2-CH_2-CH_2-COOH \quad\xrightarrow{\Delta}\quad$$

OH

δ-hydroxyacid   δ-lactone
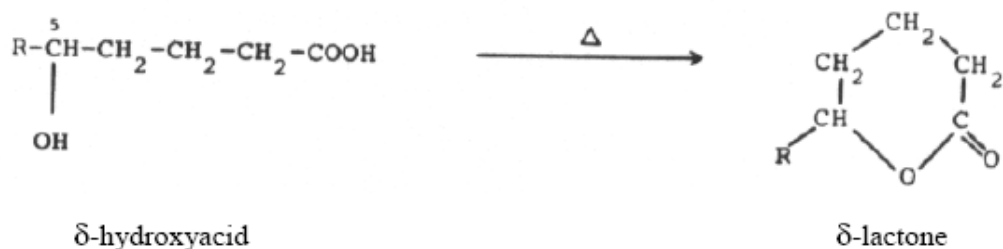
Figure 2-2. **Formation of δ-lactone from the breakdown of δ-hydroxyacid.**

7.- **Nitrogen compounds.** Nitrogen compounds do not greatly contribute to milk's aroma. However, several nitrogen compounds have been found in pasteurized milk, such as N-formylpiperidine, enzothiazole, and diphenylamine (Moio et al., 1993a).

2.2 Off-flavor compounds in milk

Milk is very susceptible to flavor defects or off-flavors due to its bland nature. Off-flavors in milk arise from five origins: (i) from the feed and/or from the cow's environment, these off-flavors are known as transmitted flavors, (ii) from chemical reactions of milk's components, (iii) from microbiological deterioration of milk , (iv) as a result of heat treatments, (v) from several origins such as packaging material, and cleaners, sanitizers, and disinfectant products. (Bassette and Fung, 1986; Saxby, 1993).

2.2.1 Transmitted flavors

Transmitted flavors are off-flavors encountered in milk, which originate in the cow's feed or the environment the cow is in. There are two ways by which transmitted flavors are conveyed into the milk: 1. from the cow's nose or mouth to the lungs then to the blood and finally to the milk; or 2. from the digestive tract to the blood then to the udder cells and finally to the milk.

The two principal feed-flavor offenders are poor quality silages and several weeds. The strength of off-flavor due to silage has been strongly associated to the moisture content of the silage and its level of ventilation. High moisture silages

are more easily accessible to mold growth and consequent spoilage. On the other hand, poor air circulation increases the concentration of volatile compounds in the air, which are carried into the milk through the cow's breath (Bassette and Fung, 1986). Certain weeds, such as wild garlic, onion and related plants are the most widespread and easily recognizable weed offenders capable of imparting milk with strong odors (Saxby, 1993). Many plants from the gramineous and cruciferae species also impart milk with off-flavors especially when present in high quantities (Urbach, 1989).

Many chemical compounds have been related to off-flavors in milk from feed origin. Mounchili et al. (2005) found that milk from cows fed baled silage presented higher concentrations of ethanol, propane-2-one, dimethyl sulphide, butane-2-one, hexanal, heptanal, and octane, than milk from cows that were not fed for the last 12 hours before milking. They did not attribute off-flavors to specific compounds but rather to the relative concentration of a common set of compounds. Similar conclusions were presented by Bendall, (2001) who in a study conducted in New Zealand on cows fed different diets (TMR diet versus pasture-feed diet) concluded that γ-12:2 lactone was the only compound to be significantly odor-active between milks.

Gordon et al. (1972) identified 18 compounds from milk characterized as feed flavored. Of these 18 compounds, 6 were held responsible for the feed flavor: methyl sulfide, acetone, butanone, isopropanol, ethanol, and propanol. However, the origin of the feed was not included in the study.

Transmitted off-flavors are also related to the environment the cow is in. Terms such as *cowy* or *barny* are commonly associated to lack of sanitization in the environment. Unsanitary conditions taint the air, produce unusual silage fermentations, and possibilities for growth of some of the bacteria responsible for off-flavors (Harper and Hand, 1976).

The incidence of transmitted flavors in milk can be minimized by controlling several factors, the most important of which is time of feeding. Generally, feed flavors are not detected if cows are milked between 4 or 5 hours after being fed. However, feed flavors may be strongly present if cows are milked only 2 or 3 hours after being fed. Ventilation is another key factor. Since odors are transmitted from the cow's lungs to the milk, it is fundamental to control the cow's environment. Finally, the cow's health may be a source of off-flavors. Several studies have suggested that the accumulation of gases, as in bloat, results in off-flavors (Bassette and Fung, 1986). Table 2-2 shows common sources of feed flavor.

2.2.2 Lipolyzed flavor

Lipolyzed flavor is one of the most common off-flavors in milk and dairy products, and is commonly described as a "rancid" flavor. This flavor defect occurs as a result of the degradation of triglycerides by the enzyme lipoprotein lipase. Heat resistant lipases from psychotropic bacteria, predominantly *Pseudomanas* species, have also been associated to lipolyzed flavors (Saxby, 1993). In the process of lipid degradation, short-chain free fatty acids (FFA)

responsible of off-flavors are released. Gonzalez-Cordova et al., (2001) associated fatty acids of C_4-C_{12} as the major contributors to lipolyzed flavors of milk. Although, lipoprotein lipase is inactivated by heat treatment, rancid flavors can not be removed once they have arisen in the milk (Saxby, 1993).

Table 2-2. Common sources of feed flavor

Sources of Feed Flavor	Sources of Weed Flavor	Feeds with Little Effect
Onions	Garlic and Chives	Sugar Beets
Silage	French weed	Dried beet pulp
Alfalfa	Mustard	Soybeans
Cabbage	Boneset	Carrots
Turnips	Buckhorn	Pumpkins
Rape	Pepper Grass	Soybean Hay
Kale	Skunk Cabbage	Potatoes
Beet tops	Ragweed	Mangoes
Green Barley	Wild Tansy	Oats
Green Alfilarei	Dog Fennel	Rye
Clover Hay	Tar weed	Peas
Distiller Grains	Alanthus Shoots	Corn
Brewer Grains		Clover and Grass
Musty hay or silage		Timothy Hay
Citrus pulp		Tankage

Jenness and Patton (1960)

2.2.3 Oxidized flavors

Oxidized flavors primarily develop during storage of milk as a result of the oxidation of polyunsaturated fatty acids (Bassette and Fung, 1986). This oxidation process brings an increase in volatile ketones and saturated aldehydes, which impart milk with an oxidized off-flavor. Perkings (2005a) found the following ketones in milk having oxidized flavor: 2-pentanone, 2-hexanone, 2-heptanone, 2-octanone, 2-nonanone, 2-decanone, and 2-undecanone; and the following aldehyedes: pentanal, hexanal, heptanal, octanal, nonanal, and decanal. Oxidation is catalyzed by copper, light, and iron. Although, copper alloys have been removed from dairy equipment, copper-induced oxidized flavor still arise in milk because milk contains a fair amount of copper. With pasteurization the susceptibility of milk to copper-induced and light-induced flavors is increased. Finally, the type of feed, homogenization of milk, and stage of lactation of the cow, have been associated to oxidized flavors (Saxby, 1993).

2.2.4 Microbial origin flavors

Microbial activity is an important source of off-flavors in any stage of the milk production process. Bassette and Fung (1986) stated that the number and sort of organisms associated with pasteurized milk are influenced by (i) time and temperature achieved during heat process, (ii) post pasteurization contamination from equipment, containers, environment, and personnel; and (iii) storage temperature and time. Thus, optimum sanitary procedures are fundamental to control the initial contamination of milk. Milk has to be quickly cooled down and

kept at 4.4°C before pasteurization to prevent microbial growth since off-flavors that arise before pasteurization cannot be removed, neither by the pasteurization process nor by vacuum treatment processes (Withfield, 1998).

Although many off-flavors occur in milk due to microbial activity, those described as acid and malty are the most commonly encountered. Acid flavors are produced during the fermentation process of lactose by *Streptococcus, Pediococcus, Leuconostoc, Lactobacillus,* and some bacteria of the Enterobacteriaceae family (Saxby, 1993). Malty flavor arises in milk as a result of the metabolic activity of *Streptococcus Lactis* subsp *maltigenum.* Several compounds such as 2-methyl propanal, 2-methyl propanol and 3 methyl propanol have been identified as being responsible for malty off-flavors (Saxby, 1993; Bassette and Fung, 1986).

2.2.5 Packaging materials as a source of off-flavors.

The primary purpose of the package is to protect the product from contamination and spoilage, however, the package itself is sometimes a source of tainting substances. Glass is considered to be virtually the only aseptic material, although contamination might occur from the enclosure material (Reineccius, 2006).   Milk bottled in glass containers is especially susceptible to photo-oxidation due to riboflavin present in milk. Riboflavin is a photosensitizer that, in presence of light, excites oxygen which reacts with unsaturated lipids forming peroxides. Several compounds have been reported as the most common compounds encountered in light-oxidized milk, such as dimethyl sulphide,

pentanal, hexanal, acetaldehyde, 1-hexen-3-one and 1-octen-3-one (Karatapanis et al., 2006).

2.3 Heat induced flavors

Milk is heated for the purpose of killing pathogenic microorganisms responsible of human related diseases, and for reducing benign microorganism with the aim to increase its shelf life (Belitz et al., 2004). The high susceptibility of milk to heat treatments causes alterations to its components. Free enzymes and whey proteins are the most heat sensitive milk compounds, whereas bound enzymes, lactose and milk lipids are the least sensitive. Heating processes modify the original flavor of the milk by giving rise to off-flavors. The degree of the modification depends on (i) temperature achieved during the heating process; (ii) time of exposure, and (iii) degree of burn-on on the heating surfaces. Off-flavors produced during heat treatments have been classified into four types: cooked or sulfurous, heated, caramelized, and scorched.

The cooked or sulfurous off-flavor is associated to the formation of volatile sulfur compounds during heat treatments above 74ºC. Volatile sulfur compounds are liberated from amino acids of milk proteins, particularly from B-lactoglobulin. These compounds are present in high concentrations right after the heating process, nevertheless, their concentration decreases over storage time (Harper and Hall, 1976). Although, pasteurized milk usually presents a slight cooked or sulfurous off-flavor (Bassete and Fung, 1986), this is not regarded as an off-flavor by many consumers due to the widespread consumption of

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pasteurized milk as opposed to raw milk (Jenness and Patton, 1960). Sulfur compounds, such as dimethyl sulfide (Vazquez-Landaverde et al., 2005), and dimethyl sulphone (Moio et al., 1993 a,b; Friedrich and Terry, 1988) have been found in pasteurized milk.

Methyl ketones have been associated to the extent of the heating process applied to the milk. Methyl ketones are naturally present in milk but are also formed during heat treatment by ß-oxidation of saturated fatty acids followed by decarboxylation reaction (Belitz et al., 2004). Several authors have intended to find a methyl compound that could be used as a marker of the degree of heat treatment. Moio and others (1993a) identified 2-hexanone and 2-nonanone as compounds present in higher concentrations in UHT milk than in pasteurized milk. Vazquez-Landaverde et al. (2005) obtained similar results but concluded that 2-heptanone, and 2-undecanone can also be attributed to the heating process. Contarini and Povolo (2002) reported 2-heptanone as the most reliable methyl ketone to use as a marker of heat treatments. Methyl ketones also increase in concentration over storage time at room temperature in UHT milks (Perkins et al., 2005b).

Aldehydes are affected to a lesser extent by heat treatments than ketones (Vazquez-Landaverde et al., 2005), although hexanal has been associated to the extent of heat treatments (Perkins, 2005b).

2.4 Sensory analysis of dairy products

Sensory properties of milk are extremely important to the dairy industry because they directly correlate product quality and consumer acceptance. Milk with sensory defects will poorly affect the quality of further processed products causing consumer rejection. The traditional approach taken by the dairy industry in relation to the sensory evaluation of milk has been to highly train judges on recognizing milk's defects and on assigning them consistent scores on a system known as score cards (Bodyfelt et al., 1998). However, this approach has been criticized for its failure to predict consumer acceptance, its lack of objectivity in quality judgments, and the complexity in assignment of quantitative scores (Chapman et al., 2001).

Discriminant analysis tests are used to differentiate among two or more milk samples (Lawless and Heymann, 1998). The most used discriminant test is the triangle test, which allows detection of small differences present among samples (Bodyfelt et al., 1988). In a triangle test, three samples are presented simultaneously to the panelist: two are the same, one is different. The panelist is asked to identify which one is the odd or different sample (Lawless and Heymann, 1998). Croissant and others (2006) conducted a triangle test on milk from feed-pasture (FP) cows against milk from cows on a total mixed ration (TMR) diet. Results showed that panelists were able to discriminate between samples, with a significance level of $p<0.10$. The same researchers conducted a descriptive analysis where FP milk was characterized as sweet, and having

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feed/malty aromas, while TMR was characterized as grassy, fecal/mothball and having higher intensities of sweet taste and sweet aromatic. Triangle tests have been conducted to evaluate thresholds of flavor defects in reconstituted whole milk powder with the aim to train sensory judges (Hough, 1992) on the sensory threshold of off-flavors caused by proteolysis and lipolysis in milk (Santos et al., 2003). Although triangle tests are widely used, their application on dairy products might present some problems as suggested by Chapman and Lawless (2005). In a triangle test of 2% versus skim milk, they found that the mean of correct responses was 54% and 53% for its replication. Using a simple binomial model, the result showed significant discrimination. However, after applying a correction for guessing, the percentage of correct discriminators dropped to 30% which was far below what was expected.

Scaling methods are used to quantify sensory attributes. The types of scales include numerical, verbal, graphical or magnitude estimation scales to quantify milk's sensory attributes. The hedonic scale, also called the 9-point hedonic scale, is primarily used for like-dislike judgments and does not require trained panelists (Lawless and Heymann, 1998).

2.5 Instrumental analysis of flavor

Identification and/or quantification of the flavor compounds present in a product is a very complicated task because none of the instruments currently available are as sensitive to odors as the human nose (Reineccius, 2006). In the

case of milk, its heterogeneous nature makes it still more complicated to properly isolate its flavor compounds (Friedrich et al., 1998).

Flavor analysis by instrumental techniques is divided into several steps. First, flavor compounds are isolated from the food matrix, and secondly identified and/or quantified. Several techniques are used for the isolation and/or concentration of volatiles compounds, including distillation methods, solvent extraction and headspace analysis, such as purge and trap (PT), static, and solid phase micro extraction (SPME) (Reineccius, 2006). The techniques commonly used for milk are vacuum distillation, purge and trap (PT), and more recently SPME. Gas chromatography (GC) is the technique most widely used for the identification and quantification of volatiles compounds (Friedrich et al., 1998).

2.5.1 Solid Phase Microextraction (SPME)

2.5.1.1 Introduction

Solid Phase Microextraction is a relatively new sample preparation technique invented by professor Janus Pawliszyn and co-workers in 1989 (Pawliszyn et al., 1997). The SPME technique does not require the use of solvents, is fast, and inexpensive. Its application requires fewer steps than traditional analytical methods, which minimizes the potential loss of analytes (Ouyang et al., 2006). Detection limits in the order of 5-50 pg/g are achieved for some volatile compounds. The sensitivity obtained with SPME depends upon several factors such as type of fiber used, volume of the sample, extraction temperature and

extraction time, introduction or not of salting-out agents, and mode of extraction (Wardencki et al., 2004).

The type of fiber used and its thickness are the two most important parameters that affect the final sensitivity of the method. Selection of fiber type depends on the polarity of the analytes, based on the rule that the polarity of the fiber attracts compounds of similar polarity. Thick fibers are mostly employed to extract high quantities of compounds, while thin fibers are used to isolate and preconcentrate substances of high boiling points. Supelco (Bellefonte, PA) is the only company that commercializes SPME devices. Table 2-3 shows the currently available fibers and their characteristics.

The SPME apparatus is a simple syringe-like device consisting of a barrel and a metal needle. The fiber is a fused silica optical material coated with a thin polymer film of 1-2 cm in length (Wardencki et al., 2004). The mode of operation is simple. First, the needle is inserted into the vial containing the sample. Secondly, the fiber is placed in contact with either the sample or the sample's headspace by pushing down the plunger. After sampling, the fiber is retracted into the needle and finally the analyte is transferred into the instrumental technique that will be used. When a GC is used, transfer is done through thermal desorption in the GC injector port. When HPLC is used, the analyte's transfer is achieved through elution by the mobile phase (Vas and Vekey, 2004).

Table 2-3. Summary of commercially available SPME fibers

Fibre Coating	Polarity	Thickness (µm)	Technique	Compounds to be analyzed
Polydimethylosiloxane (PDMS)	Nonpolar	100	GC/HPLC	Volatiles
PDMS	Nonpolar	30	GC/HPLC	Non-polar semivolatiles
PDMS	Nonpolar	7	GC/HPLC	Medium to non polar semivolatiles
PDMS/Divinylbenzene(DVB)	Semi-polar	65	GC	Polar volatiles
PDMS-DVB	Semi-polar	60	HPLC	General purpose
PDMS-DVB[a]	Semi-polar	65	GC	Polar volatiles
Polyacrylate (PA)	Polar	85	GC/HPLC	Polar semivolatiles(phenols)
Carboxen/PDMS	Semi-polar	75	GC	Gases and volatiles
Carbowas-PDMS[a]	Semi-polar	85	GC	Gases and volatiles
Carbowax/DVB	Polar	65	GC	Polar analytes (alcohols)
Carbowax/DVB[a]	Polar	70	GC	Polar analytes (alcohols)
Carbowax/templated resin	Polar	50	HPLC	Surfactants
DVB-PDMS-Carboxen[a]	Semi-polar	50/30	GC	Odors and flavors

[a] Stableflex type is on a 2 cm length fibre.

Vas and Vekey, (2004)

2.5.1.2 Principle

Once the fiber is placed in contact with the sample matrix, there is a transfer of analytes from the food matrix to the coating material. The coating material acts like a sponge attracting those analytes of similar polarity. This extraction process is completed when the equilibrium between food matrix and fiber coating is reached. This equilibrium is explained by the following equation:

$$n = \frac{K_{fs}\, V_f\, V_s\, C_o}{K_{fs}\, V_f\, V_s}$$

Where:

n is the amount of compound X absorbed by the fiber

K_{fs} is the distribution coefficient between the fiber coating and the sample matrix

V_f is the volume of the fiber coating

V_s is the volume of the solution

C_o is the initial concentration of X in the solution

Vas and Véjey (2004).

SPME is commonly used in analysis of the volatiles constituents of milk. SPME has been used to (i) analyze short-chain free fatty acids (Gonzalez-Cordoba et al., 2001), (ii) off-flavors in milk from microbial origin (Marsili, 1999), (iii) off-flavors from feed origin (Mounchili et al., 2005), (iv) off-flavors due to lipid oxidation (Perkins et al., 2005[a]), (v) oxidized off-flavors (Perkins, 2005a), (vi) sulfur compounds (Vazquez-Landaverde et al., 2006), (vii) thermally derived off-

flavors (Vazquez-Landaverde et al., 2005a), and (viii) changes in flavor volatiles as affected by packaging material and storage time (Karatapanis et al., 2006).

2.5.1.3 Disadvantages of SPME in flavor analysis

In a short time, SPME has become a widely used preparation technique due to the advantages previously mentioned (Ouyang, 2006). However, SPME has several limitations. Yang et al. (1994) pointed out the incompatibility of using external standards along with SPME for quantification of complex sample matrices. The rapid deterioration of the coating has also been reported as a problem for long term studies (Pillonel, 2001).

2.5.2 Gas chromatography-mass spectrometry (GC-MS)

GC-MS is the most commonly used technique to separate, identify and quantify volatile compounds. GC separates the components of a sample, while MS identifies those substances by their mass spectrum. After the injection of a small amount of sample into the GC, the sample is vaporized and its compounds are separated as they travel through a coated column. Separation is achieved based on each component's chemical characteristics. Then, the separated compounds enter the MS where they are bombarded by electrons to produce ion fragments. Each compound produces a specific mass spectrum, which is identified by comparison to an existing database of mass spectra. Quantification is achieved by measuring the relative intensities of the mass spectra (Gordon, 1990).
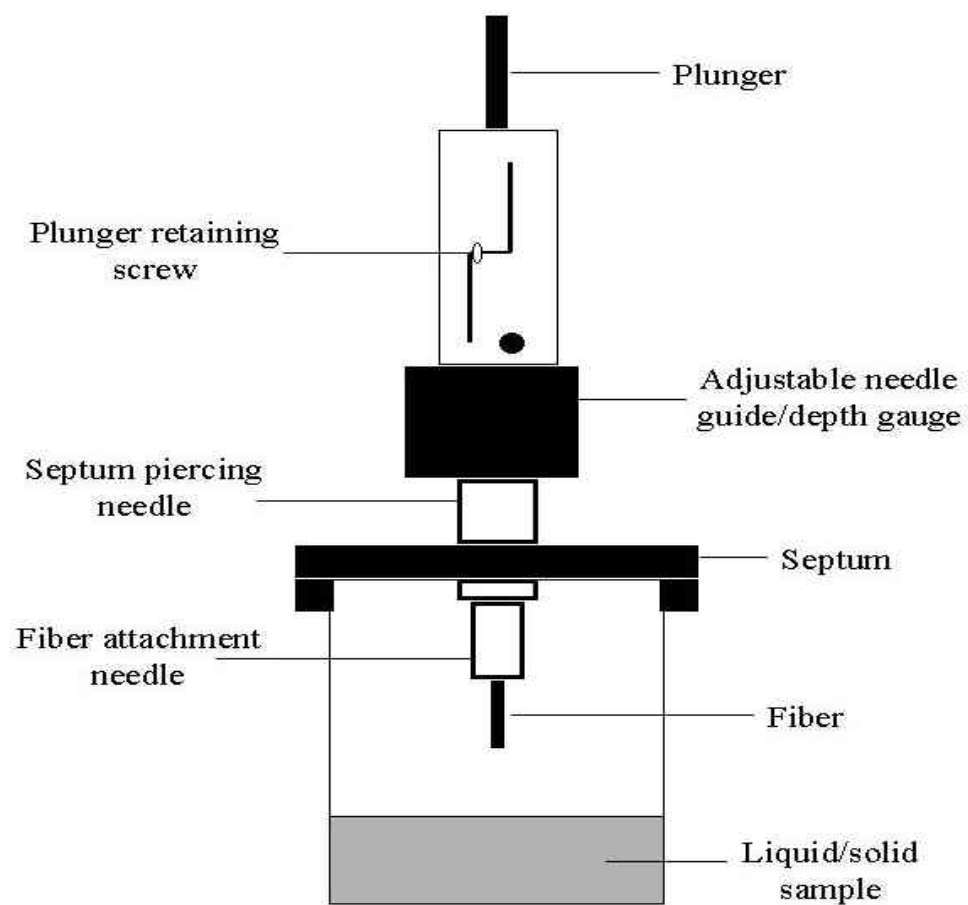
Figure 2-2. SPME device. Randolph et al. (2002)

2.6 Organic food

2.6.1 Introduction

According to the US Department of Agriculture (USDA), organic production is "a production system that is managed in accordance with the [Organic Foods Production] Act and regulations in this part to respond to site-specific conditions by integrating cultural, biological, and mechanical practices that foster cycling of resources, promote ecological balance, and conserve biodiversity" (USDA National Organic Program Standards, 2002). Organic producers and handlers subscribe to organic standards set by the National Organic Program, which addresses the methods, substances, and practices that can be used in the production, handling and manufacturing of organic products.

In general terms, the philosophy behind organic production is based on working the land and raising animals with minimal use of off-farm inputs, and the employment of respectful practices in regard to the environment, the producer, and living organisms (Benson and Kirkel, 1999).

The USA organic food industry grew 16.2% in 2005 accounting for $14.6 billion in consumer sales, a percentage that represents 2.5% of total food sales in the USA. Since 1997, the organic food market has grown extremely fast with annual growths between 15 to 21% (OTA, 2006). This growth can be explained by three main reasons. First of all, there has been an increasing introduction of organic products into the marketplace (Zepeda et al., 2004) so the array of

organic products has expanded from fruits and vegetables, once representing the whole array of organic products, to processed organic food (Dimitri and Greene, 2000). Secondly, organic foods are available to more consumers than ever before (Zepeda et al., 2004). While in its beginning the organic market was an insignificant sector with few small stores selling locally, nowadays the sector includes nationwide organic processors and distributors. At the same time, many conventional manufacturers are introducing organic items into their lines. Finally, the approval of new laws in 2002 has regulated the market facilitating transactions across the nation and internationally. In addition, there has been an increase in the amount of research conducted on organic food and an augmentation of the resources available to educate consumers about organic farming systems (Dimitri and Greene, 2000).

2.6.2 The history of organic food

The organic movement started in California in 1973 with the foundation of the California Certified Organic Farmers, the first agency to set up organic production standards. In the 8Os, due to the growing demand for organic food, other agencies opened across the country each of them setting up their own standards, which resulted in a lack of uniformity among organic products across the country (Böstrom et al., 2006). As a result, the Organic Trade Association was founded with the objective to implement a nationwide voluntary program of uniform organic standards throughout the country. However, a final agreement on the standards could no be reached. In 1990, organic retailers and producers

asked Congress to launch a mandatory organic program because they needed to facilitate commerce across the country. Two years later, the National Organic Standards Board was established with that aim. Yet, a final rule was not accepted until 2002 when the third draft was presented. The previous two drafts, in 1992 and 1997, were rejected due to strong objections among producers and retailers (Fisher, 1999).

2.6.3. Certification process

All products must undergo demanding tests in order to be certified organic. The process of certification is not done directly by the U.S Department of Agriculture for the producer, but rather it is divided in two steps. First, the National Organic Program (NOP) "accredits organic certification agencies under the authority of the Organic Foods Production Act of 1990 (7 U.S.C 6501. et seq.), as described in the Code of Federal Regulations Title 7, Part 205, National Organic Program, Final Rule" (USDA National Organic Program Standards, 2002). Secondly, these Organic Certification Agencies certify organic producer and handlers.

Currently, there are 55 domestic and 45 international accredited agencies in the USA. Certification agencies, either government or private agencies, must meet the requirements stated in NOP final rule, subpart F, §§205.51 through 205.503 in order to be certified by the NOP Accreditation Committee (USDA National Organic Program Standards, 2002)

In regard to labeling requirements, organic products are divided into 4 categories, depending on the percentage of organic ingredients present. These four categories are:

- "100 percent organic": Except for water and salt, the rest of the products must be 100% organically produced.

- "organic": Products that contain at least 95% of organically produced ingredients.

- "made with organic ingredients": Applies to those products with at least 70% of organically produced ingredients.

- Products with less than 70% of organically produced ingredients, are not allowed to use the term organic on the main display panel but organically produced ingredients may be indicated on the ingredient statement on the information panel under "made with organic".

All the certified organic products must carry the USDA logo on the packaging

Figure 2-3 USDA organic seal.

National Organic Program (2002)

2.6.4. Organic dairy farming

The organic dairy sector is the second largest organic category after fruits and vegetables. According to the Organic Trade Association's 2006 Manufacturer Survey, sales of dairy products in 2006 reached $ 2 billion, which represented a 24% growth over the previous year.

Milk is the most commonly sold organic dairy product. Its consumption has experienced an incredible growth in the past two decades thanks to its freedom of r-BGH (recombinant bovine growth hormone) (DuPuis, 2000), and to the believed superior qualities over traditional milk (Hill et al., 2002). Organic milk is milk produced according to organic standards, which address the practices and substances that can be used in milk production including livestock practices and crop production.

In regard to organic crop production, "fields are considered organic three years after the last application of a non-permitted pesticide, herbicide or chemical fertilizer. If non-permitted substances are applied to adjacent fields the farmer must construct a buffer to avoid contamination. Non-synthetic mineral amendments and manure are allowed, whereas most of the fertilizers are prohibited" (USDA National Organic Program Standards, 2002)

Regarding organic livestock practices, "the farmer must provide all animals with access to the outdoors and to pasture, clean and dry bedding, and appropriate shelter. The producer must not use drugs that promote growth, feed formulas containing urea or manure, feed mammalian or poultry slaughter by-

products, use feed or feed additives in violation of the Federal Food, Drug, and Cosmetic Act, and establish and maintain preventive livestock health practices" (USDA National Organic Program Standards, 2002). Farmers are not allowed to use synthetic medications expect those specifically included on the National List of synthetic materials allowed for use in livestock production. Antibiotics are also not permitted unless the animal's life is at risk. In this case, the animal must be either separated from the other animals or sold, but in any instance, its milk or meat cannot be labeled as organic. Vaccines, although allowed, are not required (USDA National Organic Program Standards, 2002).

2.7 Confinement systems

Confinement systems are the most used system for holding dairy cows in the USA. Confinement refers to limited quarters where cows spend most part of their time. Confinement systems can be total or partial depending on the degree of confinement. The principal advantage of confinement systems over feed-pasture and organic systems is the better feed efficiency (Ensminger, 1980).

Several comparatives studies have shown that in regard to herd's health, there are no fundamental differences between conventional and organic systems. Regarding quality of product, it cannot be stated that products differ between both systems. Both parameters herd's health and quality of the product have been primarily linked to specific farm managements rather than to the production method used. The benefits of organic products are primarily related to

environmentally friendly production and to animal welfare issues (Sundrum, 2001). No differences have been found in the gross composition, fatty acids, iodine and selenium content of milk from conventional and organic farms of similar management practices. However, urea content and somatic cells were found to be lower in organic milk, although reasons for that were not reported (Toledo et al., 2002).

2.8 Pasture-fed based system

Good pastures provide cows with all the nutrients they need and reduce the production cost of milk. In spite of that, there is a trend towards confinement production especially in big and highly specialized farms in view of the fact that confinement systems facilitate farm's automation and eliminate the cost of maintaining the land. Nonetheless, many farmers prefer to combine both systems, which is that cows are fed pasture during the warmer seasons and are in confinement the rest of the year (Ensminger, 1980).

The type of pasture varies from area to area in relation to characteristics of the soil, rainfall, and temperature (Ensminger, 1980). Pastures are typified according the type of legume. In Missouri, tall fescue is the most cultivated grass planted because it tolerates freezing temperatures (Missouri Forage and Grassland Council, 2005).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Milk samples

A total of 30 whole fat milk samples were purchased from November 2006 through February 2007 in the state of Missouri. Dairies were located in north-east Missouri (3), north-west Missouri (1), central Missouri (1), and south-east Missouri (1). Milks were obtained from three different production systems, including certified organic, pasture-feed based, and conventional. For each, two samples from two dairies were obtained and each sample was replicated five times over the four months that the study lasted. Cows from pasture-feed based systems were initially raised mostly pasture (self-proclaimed pasture-fed), however, the diet of the cows was switched to more of a conventional supplemental feeding regime upon arrival of winter time. All samples were commercially homogenized, pasteurized and bottled in glass containers except for one organic milk and one conventional milk, which were purchased raw directly from the farmer and transported to the University of Missouri in a plastic container. Samples were stored at 40°F, and analyzed before their expiration date. All milk samples came from Holstein cows except one organic milk that came from a mixture of Holstein, Jersey, and Aysrhire cows, and aconventional milk that came from a mixture of Holstein and Guernseys cows. Table 3-1 shows

the characteristics of each milk including category, samples, milk's treatments, cow's breed, and cow's feed.

Table 3-1. Characteristics of the 6 milks of the study. Category, samples, milk's treatments, cow's breed, and feed.

Category	Samples	Treatments	Breed	Feed
Organic	O1	Homogenized Pasteurized	Holstein,Jersey Ayrshire	Hay, grass, clover, barley.
	O2 [a]	Raw	Holstein	Grass, alfalfa, corn silage.
Pasture-fed	P1	Homogenized Pasteurized	Holstein	Alfalfa, hay and corn.
	P2	Homogenized Pasteurized	Holstein	Hay, wet brewers, grain.
Conventional	C1	Homogenized Pasteurized	Holstein	---
	C2 [a]	Raw	Holstein Guernseys	TMR[b]

[a] Samples were pasteurized and homogenized at the University of Missouri Dairy Pilot Plant
[b] Total Mixed Ratio. Consist of several ingredients: corn silage, alfalfa silage, corn, soy bean meal, soy hulls, brewers yeast, cotton seed.

3.1.2 Volatile compound analysis

Serum type reaction vials 10 mL (25.0 x 50.0 mm, cat. No.33105-U), open center seals (20 mm for 5-100 mL vials, Cat. No. 27230-U), septa (20 mm, PTFE/silicone, 60 mm thick, cat. No. 2-7541) and a hand crimper (Cat. No 33195) were purchased from Supelco Co (Bellefonte, PA).

Also used were an analytical balance AG 204 DeltaRange® from Mettler-Toledo Inc (Hightstown, NJ), a water bath Isotemp 205 from Fisher Scientific (St. Louis, MO), a stirrer model 4810 from Cole Parmer (Chicago, Il), and a refrigerator Roper® RT 21SKXJWOO (Benton Harbor, MI).

3.1.3 SPME device

A 2 cm 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) stable flex SPME fiber was purchased from Supelco Co. (Bellefonte, PA). Fiber was conditioned as recommended by the manufacturer.

3.1.4 GC/MS

A gas chromatography model Varian Star 3400 CX (Walnut Creek, CA) equipped with a split/splitless injector, a flame ionization detector (FID), and a DB-5 capillary column (30 m x0.32 mm i.d., x 0.25 μm (film thickness) from J&W Scientific (Folsom, CA) were used to separate and detect the flavor components of the milk. Helium was used as a carrier gar. As a mass spectrometer, a MS Varian Saturn 2000 (Walnut Creek, CA) was used.

3.2 Methods

3.2.1 Homogenization and pasteurization.

Homogenization and pasteurization of two milks (one organic and one conventional) was carried out in the Dairy Pilot Plant of the University of Missouri. The equipment consisted of a pasteurizer (PMS, PA) and a two stage

homogenizer (APV-Gaulin, PA). Pasteurization was conducted at 175 °F for 25 seconds, with a pressure of 1500 KPA in the first stage and 500 KPA in the second stage. After pasteurization, samples were bottled in glass containers and kept refrigerated until use.

3.2.2 Fat content

Crude fat content of milk samples was analyzed by Soxhlet ether extraction after acid hydrolysis at the University of Missouri Agricultural Experiment Station Chemical Laboratories.

3.2.3 Optimization of SPME parameters.

SPME parameters were evaluated to achieve the highest sensitivity with minimal artifact formation. The parameters that were taken into account were time and temperature of extraction. Sample weight, as shown by Vazquez-Landaverde (2005), did not play a significant role on the final sensitivity obtained with the SPME technique. Nevertheless, 8 mL of sample was consistently used because, as suggested by Pawliszyn et al. (1997), that amount filled just half of the vial, which allows for enough headspace and thus, extraction efficiency could be improved. Although salting-out agents are commonly introduced with the same aim, they were not considered for this study because an exhaustive literature search showed no studies with milk in which salting-out agents were used. Besides, Marsili (1999) reported that salting out agents did not

significantly improve SPME sensitivity for pentanal and hexanal, two compounds expected to be encountered in high concentrations in this study.

Three pre-experiments were conducted with the objective to optimize fiber parameters. The first approach was to equilibrate milk sample for 5 minutes at 35°C. The SPME fiber was then introduced into the headspace and allowed to equilibrate for 1 hour before volatile compounds were desorbed in the GC injector port for 10 min.

In the second approach, and with the aim to increase volatile compounds extraction, samples were stirred while being equilibrated for 1 hour at 35°C. Milk samples were allowed to cool down for 15 minutes before desorption of volatiles in the GC injector port for 10 minutes.

The last approach taken was to increase extraction temperature to 45°C and time of extraction to 3 hours. Volatile compounds were afterwards desorbed in the GC injector port for 10 minutes.

The largest number of volatiles was extracted using 3-hour extraction time at 45°C. In fact, the number of peaks obtained after only 1 hour extraction time at 35°C proved to be quite low. These results were in agreement with Vazquez-Landaverde et al. (2005) who concluded that time is the most significant factor in the extraction of volatile compounds from milk samples. However, due to time limitations, a two hour extraction time was selected. The same authors (Vazquez-Landaverde et al., 2005) suggested that temperature also plays a significant role and concluded that 35°C was the optimum temperature. This was not in agreement with Perkins (2005a), who in a comparative study of three fiber

coatings, three extraction times and three extraction temperatures, concluded that using a 2 cm 50/30 µm DVB/ Carboxen/PDMS fiber for 90 min at 40 °C was the best combination for the extraction of heat induced flavor. However, the aim of the present study was not to extract just heat-induced flavors but a wider range of volatile compounds. For that reason, and because the same temperature was successfully used by Contarini and Povolo (2002) for studying the volatile fraction of milk, 45°C was finally chosen as the optimum temperature of extraction.

3.2.4 Flavor analysis by SPME and GC/MS

Twenty five mL of milk sample was placed in a 100-mL beaker and allowed to warm up for 30 minutes at room temperature. Afterwards, 8 mL of sample was weighed out in a 10 mL serum type reaction vial, a microstirring bar was placed into the vial, which was hermetically sealed with a 20mm PTFE/silicone septa and a open center seal by using a hand crimper. Samples were placed into a 45°C water bath. The setting on the SPME holder assembly was positioned to 1 scale unit to ensure that the fiber was properly positioned in the headspace above the sample. The fiber was allowed to absorb volatiles for 120 minutes. Afterward, the setting on the SPME fiber was changed to 5 scale units and inserted into the GC injector port (250 °C). The initial temperature of the GC oven was 35°C with a hold time of 8 min. The temperature was then increased at 4°C/min until it reached 150°C. Finally, temperature was increased at 20 °C/min

until it reached 230°C which was held for 20 minutes. All samples were analyzed in duplicate.

3.3 Statistical analysis

Data was analyzed with the Statistical Analysis System (SAS V9.1, 2003) program (SAS Institute Inc., Cary, NC). Analysis of Variance (ANOVA) was performed using the General Linear Model (GLM) in SAS to determine if significant differences existed among the 6 milk samples. Orthogonal contrasts were used to estimate differences among categories of milks (contrast of organic versus milk from pasture-fed cows, organic versus conventional, and conventional versus milk from pasture-fed cows).

3.4 Sensory study

3.4.1 Samples and sample preparation

One milk sample from each category, which were used for the instrumental analysis, were also chosen for sensory evaluation. These samples were O1, P1, and C1 (table 3.1). Milk samples were kept at 40°F before the sensory analysis was conducted.

Two hours prior to the scheduled testing time, milk samples were conditioned at room temperature to allow a serving temperature that is above refrigeration temperature, as suggested by Hough et al. (1992) based on the regulations of the International Dairy Federation (IDF) Standard 99:180 Sensory Evaluation of Dairy Products.

3.4.2 Sensory tests

A preference and a discrimination test were carried out with the aim to evaluate sensory properties of the milks. The terms and conditions of both tests were previously approved by the Campus Institutional Review Board. The sensory tests were advertised via e-mail and by posting flyers around the Division of Food Systems and Bioengineering at the University of Missouri-Columbia and were conducted at the Sensory Laboratory in Eckles Hall on Tuesday March 13, 2007 from 9 a.m. to 5 p.m. (preference test) and on Thursday, March 15, 2007 from 9 a.m. to 12 p.m. (discrimination test). Prior to taking the test, volunteers were asked to read and sign a consent form (Appendix A-B). The computerized program used to conduct the sensory tests was Computense Five 4.6 (Guelph, Ontario, Canada).

3.4.2.1 Preference test

Three milk samples (one organic, one conventional, one milk from pasture-fed cows) coded with three digit random numbers were evaluated using a hedonic scale where 1 = dislike extremely to 9 = like extremely (Appendix C). The samples (approximately 35 mL each) were served in clear plastic containers with tight-fitting lids. Mineral water and crackers were provided to panelists who were instructed to eat some crackers and rinse properly between testing. A total of one hundred untrained panelists evaluated each sample monadically (one after the other) indicating the degree of overall appearance (OP), overall liking (OL), overall flavor (OF), and overall mouthfeel (OM) for each sample.

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3.4.2.2 Discrimination test

A triangle test (Appendix D) was used as the discrimination test of choice. A total of 30 untrained panelists evaluated the milk samples in three consecutive sets of triangle tests. The sample combinations in the triangle tests were: organic milk versus conventional milk, organic milk versus milk from pasture-fed cows, and conventional milk versus milk from pasture-fed cows. Samples were presented in three-digit coded plastic cups with three samples per set. Two of the samples were the same, while one was different. Panelists were instructed to identify the different one. Mineral water and unsalted crackers were provided to panelists, who were instructed to eat some crackers and rinse properly after performing each triangle test.

3.4.3 Statistical analysis

Analysis of Variance (ANOVA) and Tukey's Honestly Significant Differences (Tukey's HSD) were conducted for the analysis of sensory data to determine significant differences among the products at $p < 0.05$.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Fat content

Fat content was analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratories. Table 4.1 shows the result of the percentage of fat content for each sample and its five replications (grams fat/100 grams sample).

Table 4-1. Percentage of fat content, mean, standard deviation of milk samples

Samples	Fat content, %					Mean	Standard Deviation
	1	2	3	4	5		
O1	3.76	4.14	3.33	2.51	3.89	3.53	0.64
O2	3.58	3.74	3.46	3.33	3.39	3.50	0.16
P1	3.74	3.93	3.73	3.53	3.21	3.63	0.27
P2	3.89	3.74	2.62	3.46	3.08	3.36	0.52
C1	3.10	3.54	3.04	3.42	3.73	3.37	0.29
C2	3.58	1.52	3.68	4.56	3.89	3.45	1.14

O- organic / P- milk from pasture-fed cows/ C- conventional.

The mean percentage of fat content for all samples was sufficient to meet the minimum legal fat content established by the Food and Drug Administration (FDA) as 3.25 percent total milkfat for whole milk (21 CFR 131.110). There were no statistically significant differences ($p<0.05$) among samples.

4.2- Consumer acceptance for hedonic test

A total of 100 panelists rated 3 milks (1 organic, 1 conventional, 1 milk from pasture-fed cows) using a nine-point hedonic scale (1 = dislike extremely to 9 = like extremely). Analysis of variance and Turkey's HSD at 5% significance level were used to analyze the hedonic data set to determinate if there were significant differences for the following attributes: overall liking (OL), overall flavor (OF), overall appearance (OA), and overall mouthfeel (OM). Results are shown in Table 4.2.

Table 4.2 - Means for each attribute on the preference test.

	OL	OF	OA	OM
Organic	4.67^b	4.48^b	5.34^b	4.92^b
Pasture	5.72^a	5.71^a	5.87^a	5.91^a
Conventional	5.84^a	5.94^a	5.67^b	5.82^a

[ab] means within a column with different superscripts are significantly different at p<0.05

Organic milk was significantly (p<0.05) different from conventional milk and milk from pasture-fed cows for the attributes OL, OF, OM and also from milk from pasture-fed cows, but not conventional milk, for OA. From the results, we can conclude that panelists clearly differentiated organic milk from conventional and milk from pasture-fed cows for their liking, whereas distinction between

conventional milk and milk from pasture-fed cows was only achieved for appearance.

Tukey's HSD at 5% significance level was run to analyze the rated scores. From the results (Table 4.2) we can see that organic milk obtained the lowest mean scores for all attributes; conventional milk obtained the highest mean scores for OL and OF; and milk from pasture-fed cows obtained the highest mean score for OM and OA. Clearly, organic milk was the least liked among the samples, whereas conventional milk and milk from pasture-fed cows were rated similarly. However, since milk from pasture-fed cows was rated highest for OA, an attribute not considered central for the sensory perception of milk, we can conclude that conventional milk was slightly preferred over milk from pasture-fed cows. One possible reason of the low scores received by organic milk could be the cow's feed. Organic cows were fed with clover, hay, grass, and barley. While hay was part of the feed for all cows, as reported by Forss (1978) clover provides milk with a rather strong flavor while barley can be a source of off-flavors (Bassette and Fung, 1986). On the other hand, conventional milk could have been rated higher simply because it is probably the type of milk that the majority of panelists are used to drinking.

Considering each milk separately, organic milk's attributes were rated from highest to lowest in the following order: OA, OM, OL, and OF. Milk from pasture-fed cows had the following order: OM, OA, OL, and OF. Finally, conventional milk had this order: OF, OL, OM, and OA. It is interesting to note that the attributes were rated in opposite order for organic milk and conventional

milk, that is, for organic milk OA was scored the highest, while it was scored the lowest for conventional milk, and it was the opposite for OF. OM and OL had similar reversed orders. OF was the attribute that was rated either the highest or the lowest (high in conventional, low in organic and milk from pasture-fed cows), which suggests that OF is an attribute that plays a critical role in the sensory perception of milks.

When results are extrapolated on a 9-point hedonic scale, all the attributes for conventional and milk from pasture-fed cows fell between the terms "neither like nor dislike" to "like slightly" (from 5 to 6 on the 9-point hedonic scale), whereas organic milk fell either on "neither like nor dislike" (OA, OF, OM) to "dislike slightly" (OF). These results showed that in general panelists did not specifically like any milk, although P and C milk were preferred over organic milk. One explanation could be that the majority of panelists might have not been habitual milk drinkers and thus, they did not have a special appeal for the product, although panelists were chosen based on not being allergic to milk products and that they consumed milk regularly. It could have also been that panelists were not whole milk drinkers but preferred 1% or skim milk. Finally, and more likely, the reason could be because the milks were served at higher temperatures than milk is usually consumed. This was based on the regulations of the International Dairy Federation (IDF) Standard 99:180 Sensory Evaluation of Dairy Products and thus, higher temperature increased flavor perception causing panelists to believe that the milks' flavor was too strong.

4.3 Discrimination test

Three triangle tests were conducted to determine if 30 untrained panelists were able to differentiate between samples. The three triangle tests conducted were: (1) organic versus conventional milk, (2) organic versus milk from pasture-fed cows, and (3) milk from pasture-fed cows versus conventional milk. A significance level of P=0.01 was chosen for this test. Based on using 30 panelists, 17 would have to select correctly the sample that was different to establish significance.

Table 4.3 – Number of correct and incorrect samples obtained in the triangle test.

	Tests		
Samples	**O vs C***	**O vs P***	**P vs C**
Incorrect	10	12	17
Correct	20	18	13
Total	30	30	30
Confidence	1.000	0.998	0.834
Significance (p-value)	0.000	0.002	0.166

O = Organic sample;
C = Conventional;
P = Milk from pasture-fed cows;
* significantly different at p<0.01.

According to the results expressed in Table 4.3, significance could be established for organic vs conventional, and organic vs milk from pasture-fed cows. No significance could be established for conventional vs pasture-feed diet milk. Therefore, panelists were able to discriminate organic milk from conventional and milk from pasture-fed cows, but not milk from pasture-fed cows from conventional milk. These results correspond to the results obtained for the preference test because (1) organic milk was clearly perceived as a milk with different attributes than milk from pasture-fed cows and conventional milk, (2) conventional and milk from pasture-fed cows received similar scores and thus, discrimination between them was more difficult to achieve.

4.4 Instrumental analysis

4.4.1 - Quantitative analysis

The aroma compounds found in each of the 6 milk samples are listed in Appendices E-J. In order to estimate if there were significant differences among milk types, orthogonal contrasts were run on the 10 compounds that were commonly found in all samples. In addition, Analysis of Variance (ANOVA) was performed using the General Linear Model (GLM) in SAS to determine if significant differences existed among the 6 milk samples. The compounds found common to all 6 milks were: methylethylbenzene, 2-heptanone, pentanal, hexanal, heptanal, octanal, nonanal, decanoic acid and two unknown compounds at retention times of 3 and 15 minutes. No significant differences were found for 2 heptanone, heptanal, and the unknown compound at retention time of 15

minutes. The rest of the compounds were found to be significantly different and are listed in Table 4-4 .

Table 4.4 - Relative area counts of compounds common to all milks

TYPE	RELATIVE AREA						
					Methylethyl		
OF MILK	Hexanal	Pentanal	Octanal	Nonanal	benzene	unknown	Decanoic acid
C1	7.249^{abc}	9.261^{ab}	3.134^b	8.404^{abc}	24.291^a	31.386^{abc}	4.392^{ab}
C2	9.219^a	10.197^{ab}	5.458^a	13.100^a	21.297^a	25.901^{bc}	3.899^{ab}
P1	5.425^{abc}	3.631^b	2.116^b	3.773^c	33.014^a	37.383^a	4.262^{ab}
P2	5.266^{bc}	7.029^{ab}	2.262^b	5.221^{bc}	31.595^a	35.423^{ab}	0.867^b
O1	4.127^c	14.668^a	3.210^b	7.462^{bc}	30.405^a	21.800^c	2.796^b
O2	8.360^{ab}	10.506^{ab}	3.944^{ab}	9.414^{ab}	26.516^a	23.783^c	6.742^a

[abc] means within a column with the same letter are not significantly different.
C- Conventional/ P -milk from pasture-fed cows / O-organic.

Hexanal

In the general comparison of production methods, hexanal was found to be significantly different between C and P. More specifically, among the milks, it was also found to be significantly different between organic samples O1 and O2, and between C2 and O1.

Hexanal has been commonly found in pasteurized milk and is associated with different origins: (1) byproduct of oxidation of unsaturated fatty acids, (2) byproduct of secondary reactions (Moio et al., 1993[a,b] Imhof et al., 1993; Tosso et al., 2002; Contarini and Povolo, 2002; Marsili et al., 1999), (3) byproduct of light abuse and Cu-induced oxidation (Marsili et al., 1998), and (4) associated to cow's feed (Scanlan, 1986). Moio et al., (1993 [a]) characterized this compound as a

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powerful, penetrating, green, grassy odor and considered it, along with nonanal, as one of the most important contributors to the aroma of pasteurized milk.

Pentanal

In general, significant differences were found between O and P. In addition, the statistical analysis revealed a trend for O to be significantly different from C. In direct comparison, only P1 and O1 were significantly different.

Pentanal has been previously reported in the literature as a milk constituent formed during lipid oxidation (Moio et al., 1993ab, Toso et al., 2002; Contarini and Povolo., 2002; Marsili, 1999). The rise of pentanal has also been associated to Cu-induced oxidation as well as to light-induced changes, which principally affect glass-bottled milk stored under fluorescent light (Van Aardt et al., 2005). Pentanal has also been associated to cow's feed by Havemose et al. (2006), who found that pentanal was higher in milk from cows fed grass-clover silage than in milk from cows fed hay, which is in accordance with the results obtained in this study (see table 3.1 for cow's type of feed).

Octanal

Octanal was found to be significantly different between C and P and more specifically the two conventional samples C1 and C2. Octanal is reported to be a byproduct of lipid oxidation which has been characterized as having a floral, grass-like, and fruity aroma (Mounchili et al. 2005).

Nonanal

Nonanal was significantly different between O and P, and C and P. It has been characterized as having a fatty, tallowy aroma and has been associated to lipid oxidation.

Unknown at retention time of 3 minutes

The unknown compound at retention time of 3 minutes was significantly different between O and P, C and P, and also O versus CP (organic versus conventional milk and milk from pasture-fed cows) when O was contrasted to the two combined.

This compound was a very important contributor to milk's differences. However, it could not be identified. Its mass spectra is shown in Appendix K

Decanoic acid

This compound was not found to be significantly different among milk types in general. However, there was a significant difference between O1 and O2. Milk sample P2 from pasture-fed cows was also significantly different from O2 for this compound.

Decanoic acid has been previously reported as a milk constituent and associated with the onset of rancid flavors (Gorban and Izzeldin, 1999; Mounchili et al., 2005) tainting milk with a burnt and persistent phenolic aroma (Mounchili et al., 2005). The composition of fatty acids in milk is primarily influenced by the type of feed, and thus, higher concentrations of fatty acids have been found in

milks from cows fed diets high in unsaturated oils such as canola, sunflower, or extruded beans (Bobe et al., 2003) .

Methylethylbenzene

This compound was significantly different between C and P, but no significant differences were observed for individual samples. However, no information could be found in the literature on methylethylbenzene as a milk constituent.

In summary, we could observe that, overall, there were significantly differences between C and P. Specifically, C and P were found to be significantly different in hexanal, methylethylbenzene, octanal, nonanal and the unknown compound at retention time of 3 minutes. Among these compounds, aldehydes were the most representative chemical class suggesting different degree of lipid oxidation between C and P. Lipid oxidation in milk products is induced by light and Cu, it increases over storage time, and has also been correlated to type of feed. Urbach, (1989) stated that milk from conventionally fed cows is more susceptible to oxidation than milk from pasture-fed cows. Thus, differences in feed could also explain the significantly differences in aldehydes between C and P in this study.

Finally, organic and milk from pasture-fed cows were found significantly different in pentanal and a trend could be established as well for conventional milk. Pentanal can, therefore, be suggested as a discriminating compound for organic milk. This correlates with the results of the sensory study because

pentanal has a strong aroma, characterized as almond, pungent and malt, which might have tainted the organic milk, and thus, facilitated its discrimination from the conventional and milk from pasture-fed cows.

Finally, the unknown compound at retention time of 3 minutes, can be suggested as a discriminating compound between the three types of milk because it was the only compound to be found significantly different among all types of milk.

The results obtained in the quantitative analysis, which showed an overall significant difference between C and P, were not in agreement with the results obtained in the sensory study because panelists could not discriminate between C and P. On the other hand, the quantitative results did not show an overall significant difference for organic milk; whereas, the sensory study showed that panelist clearly perceived organic milk as significantly different from conventional milk and milk from pasture-fed cows. This could be attributed to the nature of milk's flavor. In fact, dairy researchers and flavor analysts have long recognized that the flavor of milk is very complex and that, for the most part, is the outcome of a complex balance between several volatiles compounds (Cordoba-Vallejo et al., 1998). Several authors have also pointed out that some compounds that are found to be significantly different, might not, in fact, contribute to milk's aroma, whereas compounds that are not significantly different might strongly contribute to milk's aroma (Mounchili et al., 2004).

4.4.2 Qualitative analysis.

A qualitative analysis was conducted on those compounds that were not found to be common to all milk samples. Since the objective of this qualitative analysis was to find a correlation between the analytical and the sensory study, only those milks specifically used in the sensory study were considered (O1, P1, and C1). The 21 compounds that were found to be present in at least one of the milks are shown in table 4-5.

Since the sensory study showed that panelists could clearly discriminate organic milk from conventional and milk from pasture-fed cows, but the quantitative analysis did not show significant differences among commonly found compounds, we concluded that the differences perceived in the organic milk were probably due to a set of compounds that were only present in this milk. These compounds were 3-methylbutanal, decanal, 3-methyl-1-heptene, 1-octene, toluene, acetic acid, hexanoic acid, alpha thujene, and an unknown compound at 26 minutes retention time, which are listed in table 4-6 along with their aroma identification.

Table 4-5. Qualitative analysis of compounds not found to be common to O1, P1 and C1.

Compound	Type	of milk	
	O1	P1	C1
3-methyl-butanal	+	-	-
decanal	+	-	-
3-methyl-1-heptene	+	-	-
1-octene	+	-	-
toluene	+	-	-
propyl octanoate	+	-	+
butyl caprate	+	-	+
acetic acid	+	-	-
hexanoic acid	+	-	-
alpha thujene	+	-	-
unknown (26 min)	+	-	-
2-undecanone	-	-	+
unknown (38 min)	-	+	+
1-pentanol	-	+	-
heptadecane	-	+	+
dodecanoic acid	-	+	-
unknown (2 min)	-	+	-
1-H-purin-6-amine	-	+	-
Octadecane	-	-	+
unknown (40 min)	-	-	+
2-nonanone	-	-	+

O-organic, P- milk from pasture-fed cows, C-conventional
+ present in the milk
- not present in the milk.

Table 4-6 Compounds that were exclusively found in O1 along with their aroma descriptor.

Compound	Aroma descriptor[a]
Hexanoic acid	Unpleasant chemical, caramel-like.
3-methylbutanal	Green, malty.
Acetic acid	Vinegar-like.
Decanal	Herbaceous, orange peel-like.
Toluene	Burnt, caramel-like, sulphur-like sour-like, rancid.
Alpha thujene	Soy sauce, grassy
1-Octene	N/A
3-methyl-1-heptene	N/A

[a] Friedrich and Terry, (1998); Marsili (1999), Mounchili et al., (2005), Moio et al., (1993a)

Considering that there are numerous chemicals, which are very potent odorants, and which were only found in the organic milk, it is very likely that they are responsible for the ability of the consumer and the triangle test participants to distinguish the organic milk from the other two milk types.

CHAPTER 5

SUMMARY AND CONCLUSIONS

The results indicated that there were significant differences among milks from the three different production systems, labeled organic, conventional, and pasture-fed, based on both analytical and sensory studies.

The quantitative and qualitative analysis of the volatiles by SPME and GC-MS allowed the identification of between 20 and 24 compounds in the six milks analyzed and in their five replications. Of these compounds, 10 were commonly encountered in all milk samples with aldehydes being the most representative chemical class.

The quantitative analysis showed that, overall, there were significant differences between C and P, especially in lipid oxidation products, such as hexanal, octanal and nonanal. While the type of feed can be suggested as a possible cause of these differences because aldehydes have been found to be present in higher amounts in cows fed conventional diets than in milks from pasture-fed cows, with the exception of silage, the feed for the conventionally raised cows and the pasture-fed cows during the four winter months that the samples were collected is probably not as different as it would have been during the rest of the year.

A unknown compound at retention time of 3 minutes was suggested as the compound discriminative among organic, milk from pasture-fed cows, and conventional milk because it was significantly different among the three milk

types. Pentanal was suggested as a discriminating compound for the organic milk.

Sensory studies showed that there were differences among the three milks tested. Panelists were able to discriminate between organic versus conventional milk, and organic milk versus milk from pasture-fed cows, but panelists could not discriminate between conventional milk and milk from pasture-fed cows. In the hedonic evaluation, organic milk obtained the lowest scores for all the attributes rated, whereas conventional milk and milk from pasture-fed cows were rated similarly; however, conventional milk was highly rated in overall flavor, the attribute that seemed to play the strongest influence on the milk's sensory perception. The low scores of the organic milk were attributed to the type of cow's feed, especially to clover and barley, which have been previously linked to off-flavors in milk.

A correlation was also attempted to be established between analytical and sensory results. Pentanal was suggested as the compound that might have allowed panelists to differentiate organic milk from the rest because, (1) it was present in significantly higher amounts in organic milk and, (2) it has an almond, pungent, and malt aroma that could have tainted the milk with an unpleasant flavor. Nevertheless, a common set of compounds including hexanoic acid, 3-methylbutanal, acetic acid, decanal, toluene, alpha thujene, 1-octene, 3-methyl-1-heptene, were also suggested as responsible of the differences appreciated in the sensory evaluation of organic milk.

Although analytical results showed that conventional milk and milk from pasture-fed cows were significantly different, these differences were not reflected in the sensory study. On the other hand, the differentiation of organic milk in the sensory study was not in agreement with the analytical results. One possible explanation could be that some of the compounds that were significantly different might not have, in fact, had any large impact on the milks' flavor; whereas other compounds that were not significantly different might have had a greater impact on the milks' flavor.

The objective of this study, which was to establish if significant differences existed among milks, has been accomplished. However, further studies are needed to assess these differences in greater detail. SPME has been proven to be a useful technique, and its use along with GC-O (gas chromatography-olfactometry) is recommended for these experiments. The advantage of GC-O is that the odors of the volatile compounds are characterized while the chemicals are identified simultaneously. A descriptive test is also recommended because information can be obtained on the specific milk's traits and, therefore, data from both tests can be correlated in order to identify possible discriminating compounds among samples as well as the source of potential off-flavors. Although this study allowed for the observation of definite trends in flavor differences between milks from the three production systems, additional controls, such as collecting only raw milk samples that would all be pasteurized and homogenized in the Dairy Pilot Plant, and ultimately controlling the diet of

the cows, would provide more definite answers about the specific effects of the production systems on the flavor of the produced milk.

APPENDIX A- Example of consent form for preference test.
INFORMED CONSENT TO PARTICIPATE IN THIS RESEARCH PROJECT

I, (Name_____), (Date_____) consent to participate in this research project and understand the following:

PROJECT BACKGROUND: This project involves gathering data on milk. The data will be collected for analysis and may be published. You must be at least 18 years of age to participate.

PURPOSE: The purpose of this study is to determine sensory properties of milk.

VOLUNTARY: The survey is entirely voluntary. You may refuse to answer any question or choose to withdraw from participation at any time without any penalty or loss of benefits to which you are otherwise entitled.

WHAT DO YOU DO? All participants of the consumer sensory panel will attend one session in which they taste 3 different milks. The entire participation will take only about 10 minutes.

BENEFITS: Your participation in this research project will enrich the information base. U.S. consumers enjoy the safest and most varied food supply in the world, in large part because of the great achievements of the food science research. Milk is a commonly consumed beverage by the American consumer that has gained importance because of being a good source of calcium, which may prevent osteoporosis later in life. Your participation will help in gathering important information about preferences for milk flavors.

RISKS: The expected risks are none other than those encountered in normal daily food consumption. Please do NOT participate in this study if you have a known allergic reaction to milk products, such as being lactose intolerant.

CONFIDENTIALITY: Your confidentiality will be maintained in that a participant's name will not appear on the ballot or in the published study itself. The data will only be reported in aggregate form. Score sheets will be stored for a period of three years in a locked file cabinet in the principal investigators office and then destroyed.

INJURY: It is not the policy of the University of Missouri to compensate human subjects in the event the research results in injury. The University of Missouri does have medical, professional and general liability self-insurance coverage for any injury caused by the negligence of its faculty and staff. Within the limitations of the laws of the State of Missouri, the University of Missouri will also provide facilities and medical attention to subjects who suffer injuries while participating in the research projects of the University of Missouri. In the event you have suffered injury as the result of participating in this research project, you are to immediately contact the Campus Institutional Review Board Compliance Officer at (573) 882-9585 and the Risk Management Officer at (573) 882-3735 to review the matter and provide you further information. This statement is not to be construed as an admission of liability.

Thank you for your assistance in this study. Although great strides have been made in the instrumental analysis of foods, the development of new foods still requires the human sensory response and feedback. Your efforts are greatly appreciated. If you have any questions regarding the study, please contact Dr. Ingolf Gruen at (572) 882-6746. If you have questions regarding your rights as a participant in research, please feel free to contact the Campus Institutional Review Board at (573) 882-9585.   Dr. Ingolf Gruen

Date _____   Name Printed _____   Signature

PLEASE KEEP A COPY OF THIS CONSENT FORM FOR YOUR RECORDS

APPENDIX B- Example of consent form for triangle test.

INFORMED CONSENT TO PARTICIPATE IN THIS RESEARCH PROJECT

I, (Name_____), (Date_____) consent to participate in this research project and understand the following:

PROJECT BACKGROUND: This project involves gathering data on milk. The data will be collected for analysis and may be published. You must be at least 18 years of age to participate.

PURPOSE: The purpose of this study is to determine sensory properties of milk.

VOLUNTARY: The survey is entirely voluntary. You may refuse to answer any question or choose to withdraw from participation at any time without any penalty or loss of benefits to which you are otherwise entitled.

WHAT DO YOU DO?   All participants of the consumer sensory panel will attend one session in which they taste 9 different milks in 3 sets of 3. The entire participation will take only about 20 minutes.

BENEFITS: Your participation in this research project will enrich the information base. U.S. consumers enjoy the safest and most varied food supply in the world, in large part because of the great achievements of the food science research. Milk is a commonly consumed beverage by the American consumer that has gained importance because of being a good source of calcium, which may prevent osteoporosis later in life. Your participation will help in gathering important information about preferences for milk flavors.

RISKS: The expected risks are none other than those encountered in normal daily food consumption. Please do NOT participate in this study if you have a known allergic reaction to milk products, such as being lactose intolerant.

CONFIDENTIALITY: Your confidentiality will be maintained in that a participant's name will not appear on the ballot or in the published study itself. The data will only be reported in aggregate form. Score sheets will be stored for a period of three years in a locked file cabinet in the principal investigators office and then destroyed.

INJURY: It is not the policy of the University of Missouri to compensate human subjects in the event the research results in injury. The University of Missouri does have medical, professional and general liability self-insurance coverage for any injury caused by the negligence of its faculty and staff. Within the limitations of the laws of the State of Missouri, the University of Missouri will also provide facilities and medical attention to subjects who suffer injuries while participating in the research projects of the University of Missouri. In the event you have suffered injury as the result of participating in this research project, you are to immediately contact the Campus Institutional Review Board Compliance Officer at (573) 882-9585 and the Risk Management Officer at (573) 882-3735 to review the matter and provide you further information. This statement is not to be construed as an admission of liability.

Thank you for your assistance in this study. Although great strides have been made in the instrumental analysis of foods, the development of new foods still requires the human sensory response and feedback. Your efforts are greatly appreciated. If you have any questions regarding the study, please contact Dr. Ingolf Gruen at (572) 882-6746. If you have questions regarding your rights as a participant in research, please feel free to contact the Campus Institutional Review Board at (573) 882-9585.Dr. Ingolf Gruen

Date _____   Name Printed _____ Signature

PLEASE KEEP A COPY OF THIS CONSENT FORM FOR YOUR RECORDS

Appendix C- Example of ballot sheet for preference test

<p style="color:red;">**WELCOME to our MILK RESEARCH**</p>

Instructions:

You will taste three different milk samples and evaluate each of them one after the other.

Please masticate a cracker and then rinse your mouth with water before you start the test.

Place the milk in your mouth and then rate how much you like or dislike the sample by placing a mark on the scale that best describes your opinion.

If at any time you have a question about the test or directions, please ask the lab assistant.

Question # 1 - Sample _____

How would you rate the "**OVERALL LIKING**" of this product?

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

Question # 2 - Sample _____

How would your rate the **OVERALL FLAVOR"** of this product ?

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

Question # 3 - Sample _____

How would your rate the **OVERALL MOUTHFEEL** of this product?

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

Question # 4 - Sample _____

How would your rate the **OVERALL APPEARANCE"** of this product?

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

Appendix D- Example of ballot sheet for triangle test

WELCOME to our MILK RESEARCH

You will evaluate three triangle tests. Please, follow the directions on the screen.

Directions:

1. Start with the deli cup on the left.

2. Place the milk sample in your mouth for 5 seconds.

3. Expectorate the sample into the spit cup.

4. Place a cracker into your mouth and masticate.

5. Expectorate the cracker into the spit cup and rinse your mouth with water.

6. Open the deli cup in the middle and repeat steps 2-5.

7. Open the deli cup on the right and repeat steps 2-5.

8. Circle the number of the deli cup that is different from the other two.

234   495   127

9- Repeat steps for the second and third triangle tests.

Appendix E-   Volatile compounds identified in O1.

Peak No [d]	Retention time (min)	Compound [a]	Kovats index [c]	
			Obs.	Ref.
		Ketones		
3	2.704	2 pentanone[b]		689
10	11.227	2-heptanone	891	889
		Aldehydes		
2	2.384	3 methyl butanal[b]		662
4	2.852	pentanal	698	696
9	5.833	hexanal	800	801
11	11.848	heptanal	901	900
16	17.196	octanal	1002	1006
18	21.777	nonanal	1103	1103
20	25.854	decanal	1205	1207
		Hydrocarbons		
7	4.681	3 methyl 1 heptene	762	757
8	5.456	1- octene	788	790
6	4.488	toluene	756	767
		Esters		
22	26.958	propyl octanoate[b]	1234	
24	33.801	butyl caprate[b]	1430	
12	12.94	1 methylethylbenzene	922	919
		Alkanes		
26	38.427	hexadecane[b]	1604	
		Lactones		
25	35.833	delta nonalactone	1493	
		Acids		
1	2.113	Acetic acid		601
15	16.65	hexanoic acid	991	1013
19	24.869	heptanoic acid[b]	1179	
23	31.776	decanoic acid	1369	1373

(Volatile compounds identified in O1)

Peak No [d]	Retention time (min)	Compound [a]	Kovats index [c]	
			Obs.	Ref.
		Terpenes		
13	13.39	alpha. Thujene	930	938
		Not identified		
5	3.185	unknown	710	
17	15.584	unknown	971	
21	26.427	unknown	1220	

[a] Compounds were identified using a DB-5 column. Identification was based on agreement with the reference substance on the basis of retention indices and mass spectra obtained by electron impact mass spectrometry.
[b] tentatively identified on mass spectra
[c] Obs., observed Ref., reference
[d] peak number

Appendix F- Volatile compounds identified in O2.

Peak No.[d]	Retention time (min)	Compound [a]	Kovats index [c]	
			Obs.	Ref.
		Amines		
1	2.701	2 propanamine[b]	670	
		Ketones		
5	11.225	2-heptanone	890	891
16	29.127	2 undecanone	1292	1292
		Aldehydes		
2	2.844	pentanal	696	696
4	5.807	hexanal	800	800
6	11.816	heptanal	901	903
10	17.114	octanal	1000	1006
12	21.786	nonanal	1103	1103
14	25.874	decanal	1205	1207
		Esters		
16	26.667	propyl octanoate[b]	1234	
		Alkanes		
20	38.418	hexadecane	1600	1600
		Lactones		
19	35.863	delta nonalactone[b]	1492	
		Acids		
12	24.892	heptanoic acid[b]	1180	
18	31.818	decanoic acid	1369	1373
		Aromatic hydrocarbons		
10	18.262	limonene	1025	1030
7	12.843	1 methylethylbenzene	920	919
		Not identified		
3	3.061	unknown	706	
9	15.51	unknown	970	
15	26.45	unknown	1220	
21	38.963	unknown	1641	

[a] Compounds were identified using a DB-5 column. Identification was based on agreement with the reference substance on the basis of retention indices and mass spectra obtained by electron impact mass spectrometry.
[b] tentatively identified on mass spectra
[c] Obs., observed Ref. reference [d] peak number

Appendix G- Volatile compounds identified in P1.

Peak No.[d]	Retention time (min)	Compound [a]	Kovats index [c]	
			Obs.	Ref.
		Ketones		
2	2.779	2 pentanone	684	689
7	11.348	2 heptanone	893	890
		Aldehydes		
3	2.878	pentanal	700	696
6	5.874	hexanal	801	800
8	11.891	heptanal	902	900
11	17.272	octanal	1003	1006
12	21.853	nonanal	1105	1103
		Alcohols		
5	4.717	1 pentanol	763	764
		Alkanes		
18	38.47	hexadecane	1604	1600
21	39.769	heptadecane	1705	1700
		Lactones		
16	35.943	delta nonalactone	1496	1494
		Acids		
14	24.908	heptanoic acid [b]	1180	
15	31.876	decanoic acid	1372	1373
17	37.813	dodecanoic acid [b]	1574	
		Aromatic hydrocarbons		
9	12.925	1 methylethylbenzene	922	919
		Miscellaneous		
1	2.366	unknown	607	
4	3.095	unknown	707	
10	15.626	unknown	972	
19	38.999	unknown	1644	
22	40.982	1 H Purin, 6 amine	1836	

[a] Compounds were identified using a DB-5 column. Identification was based on agreement with the reference substance on the basis of retention indices and mass spectra obtained by electron impact mass spectrometry.
[b] tentatively identified on mass spectra
[c] Obs., observed Ref., reference
[d] peak number

Appendix H- Volatile compounds identified in P2

Peak No.[d]	Retention Time (min)	Compound [a]	Kovats index [c]	
			Obs.	Ref.
		Amines		
1	2.713	2 propanamine [b]		672
		ketones		
6	11.211	2 heptanone	889	891
		Aldehydes		
2	2.835	pentanal	697	696
5	5.819	hexanal	801	800
7	12	heptanal	900	900
10	17.176	octanal	1006	1002
11	21.759	nonanal	1102	1102
14	25.854	decanal	1207	1205
		Esters		
16	29.1	propyl octanoate [b]		1292
12	25.314	methyl salicylate [b]		1191
		Alcohols		
4	4.645	1 pentanol	764	760
		Acids		
13	25.643	benzoic acid*		1199
17	31.723	decanoic acid	1373	1368
		Alkanes		
18	38.399	hexadecane	1600	1599
19	39.718	heptadecane	1700	1700
20	40.669	octadecane	1800	1799
		Aromatic hydrocarbons		
8	12.815	1 methylethylbenzene	919	919
		Not identified		
3	3	unknown	705	
9	15.521	unknown	970	
15	26.42	unknown	1220	
21	40.782	unknown	1911	

[a] Compounds were identified using a DB-5 column. Identification was based on agreement with the reference substance on the impact mass spectrometry.
[b] tentatively identified on mass spectra
[c] Obs., observed Ref, reference [d] peak number

Appendix I- Volatile compounds identified in C1

Peak No.[d]	Retention time (min)	Compound [a]	Kovats index [c]	
			Obs.	Ref.
		Ketones		
2	2.718	2-pentanone [b]		689
7	11.266	2 -heptanone	891	889
12	21.265	2 nonanone	1091	1091
		Aldehydes		
3	2.867	pentanal	700	696
6	5.856	hexanal	801	801
8	12	heptanal	902	900
11	17.208	octanal	1002	1003
13	21.806	nonanal	1104	1103
		Acids		
1	2.219	acetic acid		
		heptanoic acid [b]		
14	24.931		1181	
15	31.851	decanoic acid	1370	1373
		Alkanes		
18	38.419	hexadecane	1600	1600
20	39.721	heptadecane	1700	1700
22	40.674	octadecane	1800	1800
		Lactones		
16	35.878	delta nonalactone	1495	1494
		Aromatic hydrocarbons		
9	12.985	1 methylethylbenzene	923	919
		Not identified		
4	3.013	unknown	705	
10	15.584	unknown	971	
19	38.95	unknown	1640	
21	40.579	unknown	1789	

[a] Compounds were identified using a DB-5 column. Identification was based on agreement with the reference substance on the impact mass spectrometry.
[b] tentatively identified on mass spectra
[c] Obs., observed Ref., reference
[d] peak number

Appendix J- Volatile compounds identified in C2

Peak No.[d]	Retention time (min)	Compound [a]	Kovats index [c]	
			Obs.	Ref.
		Ketones		
4	11.215	2 heptanone	891	891
10	21.205	2 nonanone	1090	1090
15	29.019	2 undecanone	1290	1292
		aldehydes		
1	2.848	pentanal	697	696
3	5.813	hexanal	800	800
5	11.802	heptanal	901	903
8	17.159	octanal	1001	1006
11	21.708	nonanal	1101	1103
		Esters		
13	25.755	n-propyl benzoate [b]	1201	
14	26.871	propyl octanoate [b]	1232	
17	33.666	butyl caprate [b]	1426	
		Alkanes		
19	39.589	heptadecane	1690	1700
21	40.7	octadecane	1802	1800
		Acids		
12	24.796	heptanoic acid	1178	
16	31.693	decanoic acid	1367	1373
		Aromatic hydrocarbons		
6	12.747	1 methylethylbenzene	919	919
9	18.213	limonene	1026	1030
		Not identified		
2	2.93	unknown	702	
7	15.51	unknown	970	
18	38.936	unknown	1622	
20	40.527	unknown	1784	

[a] Compounds were identified using a DB-5 column. Identification was based on agreement with the reference substance on the impact mass spectrometry.
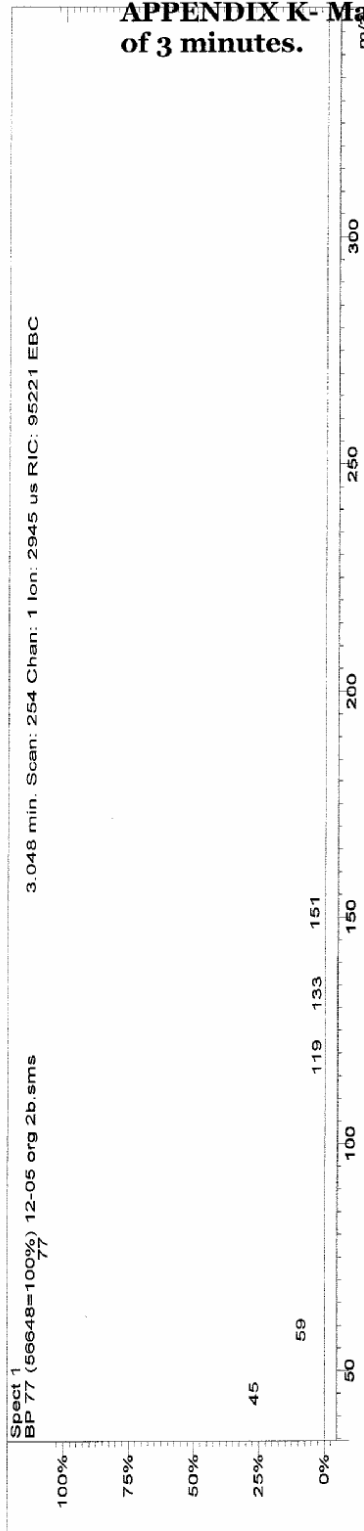[b] tentatively identified on mass spectra
[c] Obs., observed Ref., reference
[d] peak number

Scan 254 from c:\laura\12-05 org 2b.sms

Spect 1
BP 77 (56648=100%) 12-05 org 2b.sms   3.048 min. Scan: 254 Chan: 1 Ion: 2945 us RIC: 95221 EBC

APPENDIX K- Mass spectra of unknown compound at retention time of 3 minutes.

Spectrum from c:\laura\12-05 org 2b.sms
Scan No: 254,   Time: 3.048 minutes
No averaging, Background corrected (E)
Comment: 3.048 min. Scan: 254 Chan: 1 Ion: 2945 us RIC: 95221 EBC
Pair Count: 32   MW: 0   Formula: 0   CAS No: None   Acquired Range: 35 - 350

Ion	Int	%BP	Ion	Int	%BP	Ion	Int	%BP	Ion	Int	%BP
38	64	0	47	1091	2	60	281	0	76	1729	3
40	715	1	48	82	0	61	1377	2	77	56648	100
41	173	0	51	50	0	62	2383	4	78	35531	6
42	265	0	53	161	0	63	862	2	79	2204	4
43	446	1	55	123	0	64	160	0	119	211	0
44	1392	2	57	237	0	73	39	0	133	197	0
45	13659	24	58	535	1	74	243	1	151	751	1
46	925	2	59	3485	6	75	1192	2	152	110	0

72

70

APPENDIX L- SAS program for GLM analysis (ANOVA).
FAT CONTENT ANALYSIS

```
options ls=100 ps=70;
data one; infile 'f:data.csv' dsd firstobs=1 missover;
length chem$20  trt$20;
input chem$ a1a p1a a1b p1b a2a p2a a2b p2b a3a p3a a3b p3b
a4a p4a a4b p4b a5a p5a a5b p5b trt$;
*proc print;

data two; set one;
value=p1a; rep=1; det=1; output;
value=p1b; rep=1; det=2; output;
value=p2a; rep=2; det=1; output;
value=p2b; rep=2; det=2; output;
value=p3a; rep=3; det=1; output;
value=p3b; rep=3; det=2; output;
value=p4a; rep=4; det=1; output;
value=p4b; rep=4; det=2; output;
value=p5a; rep=5; det=1; output;
value=p5b; rep=5; det=2; output;
drop a1a p1a a1b p1b a2a p2a a2b p2b a3a p3a a3b p3b
a4a p4a a4b p4b a5a p5a a5b p5b ;

proc sort; by chem;
*proc print;
proc glm; by chem; class trt rep;
model value=trt rep(trt);
test h=trt e=rep(trt);
means trt/lsd lines e=rep(trt);
lsmeans trt/s p e=rep(trt);
run;
```

```
options ls=100 ps=70;
data one; infile 'i:data.csv' dsd firstobs=1 missover;
length chem$20  trt$20;
input chem$ a1a p1a a1b p1b a2a p2a a2b p2b a3a p3a a3b p3b
a4a p4a a4b p4b a5a p5a a5b p5b trt$;
if _n_=2 or _n_=12 or _n_=22 or _n_=32 or _n_=42 or _n_=52
then chem='un1';

proc print;
data two; set one;
value=p1a; rep=1; det=1; output;
value=p1b; rep=1; det=2; output;
value=p2a; rep=2; det=1; output;
value=p2b; rep=2; det=2; output;
value=p3a; rep=3; det=1; output;
value=p3b; rep=3; det=2; output;
value=p4a; rep=4; det=1; output;
value=p4b; rep=4; det=2; output;
value=p5a; rep=5; det=1; output;
value=p5b; rep=5; det=2; output;
drop a1a p1a a1b p1b a2a p2a a2b p2b a3a p3a a3b p3b
a4a p4a a4b p4b a5a p5a a5b p5b ;

proc sort; by chem;
proc print;
proc glm; by chem; class trt rep;
model value=trt rep(trt);
contrast 'o vs c' trt -1 -1 0 1 0 1/e=rep(trt);
contrast 'o vs p' trt -1 -1 1 0 1 0/e=rep(trt);
contrast 'c vs p' trt 0 0 -1 1 -1 1/e=rep(trt);
contrast 'o vs cp' trt 2 2 -1 -1 -1 -1/e=rep(trt);
contrast 'o1 vs o2' trt -1 1 0 0 0 0/e=rep(trt);
test h=trt e=rep(trt);

lsmeans trt/s p e=rep(trt);
run;
```

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