FLAVOR CHANGES IN STORED EXTENDED SHELF-LIFE FLAVORED MILKS

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By
DEENA RUTHANNE STROHMAN

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Gary Reineccius, Advisor
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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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Chapter I: LITERATURE REVIEW

When given a choice, a child will ordinarily pick a sweet, non-nutritive beverage rather than a glass of milk, while parents and health advocates wish it were the reverse. Children tend to choose that which tastes good over that which is good for them. Because of this concern, food scientists aim to make healthy choices, such as milk, more desirable. Therefore, reviewing milk and its flavor variability will give a basis to the research of the extension of desirable added flavors in milk that will be favored by children.

Flavored (chocolate, strawberry, vanilla, banana) milks are available in many markets. Kids drink them more readily than unflavored milk. Articles have been written about microorganisms found in some chocolate milk after pasteurization as well as about the flavors in strawberry yogurt drinks, but few to no articles have been written about the stability of flavor in flavored milks. Due to an absence of articles on the flavors in strawberry flavored milk, this review will examine off-flavor influences in unflavored milk.

Comparison of Consumption of Soda and Milk

Growing children are in need of vitamins, minerals, and high quality proteins to build strong bones and healthy bodies. Fluid milk is a good source of these nutrients (Committee on School Health, 2004). Although milk used to be a staple in children's diets, overall beverage milk consumption decreased 13% between 1982 and 2007 (27 gallons per capita to 24 gallons per capita) (USDA/ERS). Instead of choosing nutrient dense beverages such as milk, young people are drinking 77% more sodas (an increase from about 22 gallons per capita in 1970 to 39 gallons per capita in 1995) (Committee on School Health, 2004; Mathematica Policy Research, 2001; United States Department of Agriculture, 1997). While flavored milk consumption increased by 51% (1.2 gallons per capita to 1.7 gallons per capita) between 1986 and 2006, this was small in absolute numbers compared to soda consumption (United States Department of Agriculture, 1997; USDA/ERS). This reveals a window of opportunity for those concerned with child nutrition: improve the flavor and availability of flavored milk to compete with soda as a beverage for children or increase the nutrient value of soda drinks.

Current Milk Processing Methods

Milk is pasteurized to extend palatability and ensure safety. The primary hazard to be considered with milk is viable pathogens. According to the Pasteurized Milk Ordinance, milk should contain no more than 20,000 colony forming units (cfus/ml) (standard plate count) or 10 coliforms/ml after pasteurization (US Dept. of Health and Human Services, 1999). For many years, batch pasteurization was used to ensure safety. This Low Temperature Long Time (LTLT) process involves heating the milk for 30 min at 63ºC (Teknotext, 2003; US Dept. of Health and Human Services, 1999). Later, High Temperature Short Time (HTST) processing came into usage, which entails heating for 15 sec at around 72ºC (Teknotext, 2003; US Dept. of Health and Human Services, 1999). Now, flavored milks (often packaged in Polyethylene Terephthalate (PETE) or Polyethylene (PE) containers) are processed by Higher Heat Shorter Time (HHST) methods starting at about 89ºC for 1 sec (Teknotext, 2003; US Dept. of Health and Human Services, 1999). Ultra High Temperature (UHT) treatment methods involve heating milk to 135-140ºC for a few seconds (Teknotext, 2003). The HHST methods produce extended shelf life (ESL) milk.

Normal pasteurized milk has a shelf life of about 14 days (Marsili and Miller, 2001), while ESL milk lasts closer to 50-60 days (Wolke, 2002; Teknotext, 2003). This is due in part to the extent of the kill or reduction in spoilage organisms. Regular pasteurization is intended to eliminate coliform bacteria, typhus bacteria, tuberculosis bacteria, and phosphatase enzyme (Teknotext, 2003). LTLT pasteurization methods were created to reduce the tubercle bacteria in milk, while HTST methods were developed to deal with *Coxiella burnetti* (Teknotext, 2003; Anon, 1957; Enright et al., 1957). ESL processing also eliminates peroxidase and many heat resistant micrococci (Teknotext, 2003).

Milk Composition

Whole milk is composed of approximately 87% water and 13% solids (Teknotext, 2003). The total solids are made up of fat (4% of milk), proteins (3% of milk), lactose (5% of milk), and minerals (1% of milk). People beyond preschool age are urged to consume reduced fat milk. Reduced fat milk is composed of 89% water, 2% fat, 3% proteins, 5% lactose, and 1% minerals (USDA nutrient database). Due to the reduction of fat, fat soluble vitamins A and D3 must be added to reduced-fat milk according to the Pasteurized Milk Ordinance (US Dept. of Health and Human Services, 1999).

Flavors in Unflavored Milk

Many food products are identified by their distinctive flavors. In contrast, milk is best known as having a bland flavor or having no predominate flavor characteristic. Unflavored milk, therefore, does not lose pleasant flavors over time, but rather gains unpleasant flavors.

Due to the high water and nutrient content of milk, psychrotrophic bacteria (bacteria that grow at refrigeration temperatures) can wreak havoc on the flavor by digesting the nutrients and by the byproducts they release. Amino acids such as phenylacetic acid, leucine, methionine, cysteine, and tryptophan are broken down into pheylacetaldehyde, *iso*-valeraldehyde, methional, dimethyl disulfide, and indole, respectively (McGorrin, 2001). Saturated fatty acids break down into fatty acids such as acetic, butyric, and hexanoic acid. Unsaturated fatty acids degrade into aldehydes, lactones, and methyl ketones through oxidation and hydrolysis (McGorrin, 2001). Lactose and citric acid break down to lactic acid, acetic acid, acetaldehyde, diacetyl, acetoin, alcohols, and esters. Lastly, sugars convert to furans, furanones, and maltol through caramelization. All of these degradation products originating from fat, protein, and sugar can adversely affect flavor as we will see in detail.

Microbial Off-flavors

Microbial deterioration can cause off-flavors such as malty, acid, fermented, and unclean notes in unflavored milk (Heer et al., 1995). Malty and acid off-flavors are often caused by *Streptococcus lactis*, *Streptococcus cremoris,* or *Lactobacillus lactis*. These microorganisms are a problem with delayed cooling of milk. Fermented and fruity flavors are often caused by *Pseudomonas fragi*, which is a problem when raw milk is stored too long before pasteurization. Bitter and unclean off-flavors may be due to psychrotrophic bacteria, which are a problem if the milk is not kept at a low enough temperature. In addition, the microorganisms present in milk can vary throughout the year. For example, San Buenaventura et al. (1991) found that milk in winter was described as having a bitter off-flavor (probably caused by microorganisms) while milk in summer was often described as rancid (probably caused by enzymes).

Microbial growth is not only a flavor issue, but also a safety issue. Bishop and Bodine (1986) measured standard plate count, coliforms, flavor score, psychrotrophs, gram negative bacteria, and lipopolysaccharide (LPS) concentration in pasteurized whole milk stored at 7°C for 0 to 21 days. Only LPS and flavor score consistently increased with time although all except coliform count increased overall (from 0 to 21 days). LPS relates to byproducts of growing bacteria (Bishop and Bodine, 1986). LPS concentration correlated ($p<0.01$) with psychrotrophic count ($r=0.7$), flavor score ($r=0.9$) and days of storage ($r=0.9$). Marsili and Miller also hypothesized that the flavor has to do with both the type of bacteria present and native enzymes (such as lipases and proteases) still active after pasteurization, and not necessarily the psychrotrophic plate count (Marsili and Miller, 2001; Cadwallader and Howard, 1998). *Listeria* and *Yersinia* (psychrotrophic bacteria) could be a problem if there is post-pasteurization contamination, as could *Bacillus cereus* spores that are the only ones that can survive pasteurization and can sometimes grow at refrigeration temperatures (Chaintreau, 1999; Muir, 1996; Chapman et al., 2001). Wong et al. (1988) found 2% each of pasteurized and fermented milks in China contained *Bacillus cereus*, 98% of which could grow verotoxin. Dommett et al.

(1994) found *Pseudomonus florescens* in milk with high aerobic plate counts. In other words, even pasteurized milk can contain inactivated enzymes and harmful bacteria.

Absorbed Off-flavors

Absorbed off-flavors are transmitted to the milk while it is still in the cow or through packaging during storage. Taints are off-notes from contamination, while off-flavors are off-notes from product degradation. Three main types of cow related absorbed off-flavors come from the feed (compounds in the cow's food), the barn (volatiles inhaled by the cow), and the cow itself (compounds caused by physiological malfunctions in the cow) (Heer et al., 1995). Burton (1983) divided off-flavors into two groups: those not related to storage (such as feed and weed taints, taints picked up from things stored nearby, taints from packaging, and flavors and astringency from heating) and those developed during storage (off-flavors caused by bacteria, enzymatic activity, light, lipid oxidation, or oxidized Maillard reactions). Both taints and off-notes are involved in the degradation of milk flavor.

Biochemical/Chemical Off-flavors

Biochemical and chemical reactions can cause rancid and off-flavors in milk. Rancid off-flavors originate with hydrolysis of milk fat by lipase (Heer et al., 1995). Pseudomonads are the main lipolytic psychrotrophs found in raw and UHT milk (McKay and Beacham, 1995). McKay and Beacham added LS107d2 (a lipolytic pseudomonad) to whole milk and monitored it for rancidity by following the free fatty acids concentration over time and at different temperatures. Free fatty acid levels increased over time and then decreased (production less than consumption). Oddly, free fatty acid levels were greatest at 25°C, lower at 30°C and 40°C, and lowest at 33°C. Light oxidized flavors come from the auto-oxidation of lipids (under the influence of light) and the subsequent breakdown of sulfur containing amino acids (Heer et al., 1995). Kim and Morr (1996) studied light-exposed milk and found that 2-butanone and 2-propanal increased quickly after 12 hrs of light exposure and then remained constant; this increase was drastically affected by the headspace left in the test tubes. With no headspace, these compounds increased slowly only after 24 hrs. The same observations were made for pentanal and hexanal. On the other end, the dimethyl disulfide measurements did not show conclusive trends. Many other compounds were formed and their formation mechanisms were reviewed by Cadwallader (Cadwallader and Howard, 1998).

Processing Off-flavors

Processing off-flavors include cooked, flat, and foreign type notes (Heer et al., 1995). Cooked flavor is caused by sulfhydryl compounds that formed when milk is heated at high temperatures or for a long time. For example, heating milk to 90°C for 30 min rather than for 10 min triples the measured hydrogen sulfide concentration (Boyd et al., 1957). Gould and Sommer found that heating milk to between 76°C and 78°C (with a 15 min come up time) produced cooked flavor and sulfhydryls (1939). Jaddou et al. (1978) determined that β-lactoglobulin in whey forms reactive sulfhydryl groups. Jaddou found that concentrations of H_2S, CH_3SH, and CS_2 in UHT milk as well as cabbage-like off-flavors decrease together. Foreign flavors are caused by contamination with foreign substances such as sanitizers, detergents, and medications as was exemplified by Jeng et al. (1988). This research group investigated the link between short chain aldehydes (acetal, propanal, n-pentanal, and n-hexanal) and copper in laboratory pasteurized milk. They found that adding 5ppm of cupric sulfate increases n-hexanal concentration the most (0 ppb versus 1914 ppb in the control versus treated sample, respectively) then n-pentanal (619 ppb when copper was added).

Flavor of Flavored Milk

Flavored milks usually start with a pleasant flavor. The development of an off-flavor may be due to an increase in unpleasant flavor (like in unflavored milk), a decrease in pleasant flavor, or a combination of these two phenomena. This being said, little research has been conducted on the flavors of flavored milk. Antoine and Donawa (1990), Evenson et al. (1988), and Jensen et al. (2001) identified sources of microbiological deterioration in spoiled chocolate milk. Pearson and Marth (1990) found that the addition of cane sugar and cocoa powder to skim milk increases the growth of *Listeria monocytogenes* by approximately one log cfu/ml in 30 h of growth time with incubation at 30°C. In addition, Douglas et al. (2000) found that milk pasteurized with cocoa powder and sucrose, or with cocoa powder without sucrose had significantly greater growth of microorganisms after storage for 14 and 21 days than did milk with or without added sucrose. However, none of these examined the overall milk flavor (especially in non-spoiled milk).

Strawberry Flavor

Strawberry flavor has a variety of instability issues in non-milk drinks. Scanlan et al. (1965) and Golaszewski et al. (1998) identified (Z)-3-hexenal, 4-hydroxy-2,5-dimethyl-3(2H) furanone, 2,5-dimethyl-4-methoxy-3(2H) furanone, and methyl butanoate as key aroma compounds in strawberry juice. Siegmund et al. (2001) found that the intensity of aroma of a strawberry drink decreased over time in storage at 27°C. Van Aardt et al. (2001) found that the flavor threshold for an undesirable flavor was significantly greater in chocolate milk than in spring water.

Sensory Analysis

Instrumental methods may give accurate information regarding chemical changes over time, but that does not give information regarding the perception of flavor. For that, we need sensory input. Descriptive analysis can help us to characterize flavor attributes and describe flavor changes over time. In descriptive analysis, a trained panel is presented with samples and asked to rate the intensity of flavors, colors, textures, or other sensory attributes using a line scale (Lawless and Heymann, 1999). During training, the panelists are given a representative sampling of products and asked to determine a set of words to describe the differences between these samples. In the related training sessions, panelists are asked to narrow down terms until they have enough to describe the flavors present without having two terms addressing one characteristic (Civille and Lawless, 1986). Determining the panelist reproducibility is important to ensure that the panelist is consistent in choosing responses as a descriptive panel is considered a calibrated instrument (Lawless and Heymann, 1999).

Although one may have both instrumental and descriptive sensory data on milks during storage, hedonic tests are needed to determine the actual acceptability of these milks. Liking can be determined by tasting milk samples and rating them on a hedonic scale. A common structured 9-point scale lists choices ranging from "dislike extremely" to "like extremely" (Lawless and Heymann, 1999). Scales for children sometimes have fewer choices or different words. For example, the P& K Verbal Scale for Use with Children has anchors ranging from "super bad" to "super good" (Kroll, 1990). Although descriptive panels are considered trained instruments, liking panels are more similar to opinion polls and, therefore, require more participants to be statistically valid. As an example, Heer et al (1995) correlated liking of milk with different milk flavors, but unexpectedly did not find related differences in liking.

Much of the research we found on flavored milks had to do with milkshakes. Quiñones et al. (1997) showed that increasing total protein content and total fat content improved the appearance or visual measures of viscosity as well as mouth coating and thickness texture ratings, however, fat changes were more significant. Yanes et al. (2002) found that commercial chocolate milks with different (unspecified) fat levels (but similar levels of soluble solids and pH) varied widely in measured viscosity between products and even between lots of the same product. Holsinger et al. (1987) studied the hedonic ratings of chocolate-flavored milkshakes, first optimized with ratings by college age people, then tested on their target milkshake audience, who were high school students.

Milk is often rated using dairy judging protocol. In one study, Claassen and Lawless (1992) attempted to compare the sensitivity of descriptive analysis terminology with the sensitivity of dairy judging terminology. The panel trained in descriptive analysis found differences in paired comparisons 93% of the time (when samples were different) while the panel trained in 3 dairy judging terms (encompassing many aspects of a certain defect) found differences only 73% of the time. Lawless and Claassen (1993) found the descriptive analysis panel the same as or better than the trained dairy judging terminology panel at finding light, metallic, and rancidity differences in prepared milk. They also found convenience samples and random samples gave similar liking ratings for chosen milks. Convenience samples include participants who are readily available (Cobb, 1998). Random samples give all possible participants an equal chance of being chosen. Most actual samplings of human population are a compromise between random and convenience samples. We will include both a descriptive analysis panel of adults and a liking panel of children (the target audience) to address the hypotheses below.

Instrumental Sampling

A technique called Solid Phase Microextraction (SPME) is a way to isolate and inject milk volatiles without adding protein and fat. SPME is a fast, solvent-less, and economical way to measure the volatiles in milk (or other) samples (SUPELCO catalog, 2003). SPME uses a fiber with a fused silica core coated with a thin layer of absorptive material to extract volatiles from the headspace of samples (Wercinski and Pawliszyn, 1999). SPME requires little to no pretreatment of the sample and can be automated. However, SPME may not be as sensitive as Purge and Trap and Solid Phase Extraction methods (Penton, 1999). Fiber choice is a concern as the fiber chosen will determine which compounds are collected (Shirey, 1999).

SPME fibers, however, give researchers many options in terms of trapping specificity (Shirey, 1999), one being polarity. In a study of milk off-flavors Marsili has recommended using a 75 μm Carboxen/Polydimenthylsiloxane (PDMS) fiber (Marsili, 1998). Carboxen/PDMS has a combination of PDMS (nonpolar) and Carboxen (polar) phases (Shirey, 1999). Another choice is porosity. Carboxen/PDMS has evenly distributed multi-sized pores. Different sampling temperatures can vary the amount of compounds absorbed by the fiber, with less volatile compounds being captured better at higher temperatures. SPME fibers are sensitive enough to pick up compounds at the low ppb range, but can be overwhelmed if large amounts of compounds are present. Carboxen/PDMS fibers select well for acids (C2 to C8), alcohols (C1 to C8), aldehydes (C2 to C8), ethers (C4 to C12), hydrocarbons (C2 to C10), ketones (C3 to C9), and sulfur gases mostly in the 1 ppb to 1 ppm range. The PDMS phase is used for monitoring gases and low molecular weight compounds (MW 30-225) (SUPELCO catalog 2003). A special SPME liner can be used to quickly move volatiles onto the column in a narrow band. The SPME liner has a 0.75mm inner diameter compared to a 2mm inner diameter found in most Gas Chromatograph (GC) inlets. Although GC techniques are objective and may measure compounds down to a 10^{-13} concentration, there is no assurance that they will measure compounds that are important to determining the liking of the milk or even the perception of flavor of milk.

Given the above, we will use SPME to sample the flavored milk because it is fast and does not require solvent. We will later couple the GC with Mass Spectrometric (MS) capabilities in order to better identify compounds in milk and determine how they change over time.

Statistics

Statistics are a very important consideration in experiment planning. To control for bias, randomization can be used (Ohlert, 2000). For randomization, samples are listed and assigned numbers from a random number table. The samples are then analyzed in order from the lowest to the highest assigned number. The instrumental method (GC) we used for the study has the ability to analyze 32 samples in one "session", so we could randomize samples in blocks of up to 32. When more than 32 samples need to be analyzed, we will use randomized complete block designs to ensure that all samples are evaluated at least once next to every other sample. Human panelists would probably not be able to taste even 24 samples in one session without a carry-over effect, especially since many of the samples are stored for a number of weeks and can contain undesirable flavors (Lawless and Hildegarde, 1999). A series of designs developed by Williams in 1949 can be used to balance the order of treatments given within a block (Macfie et al., 1989). This Latin square type method varies the order of treatment presentation along with the pairs of treatments presented together. Although not every panelist would receive every order of treatments, overall, the group of panelists would receive all of the different presentation orders.

Hypotheses

Based on the literature review, more research is needed on the stability of flavors in flavored milk. Our project requires that we have some means of monitoring flavor changes in flavored milks over time that provide both a flavor quality assessment (sensory method) and a means to determine underlying causes for observed quality changes (instrumental method). The instrumental method of choice is gas chromatography/mass spectrometry (GC/MS) with SPME as the extraction method. While GC will permit only measuring changes in the volatile components of milk, it appears to be the best choice available. For sensory methods, we will primarily use a descriptive analysis panel in concert with a study of liking of the milk by children.

Plain and chocolate milk have been studied whereas strawberry flavored milk has not. Plain milk usually becomes undesirable with time due to the degradation of major milk flavors. We will determine if strawberry flavored milk experiences the same degradation of milk flavors. Further, we will determine the flavor addition of strawberry flavor over time. Parameters of hypotheses 1 and 2 will be decided and described in chapter 2.

1. Strawberry flavored milk becomes more undesirable with time due to the degradation of strawberry flavor (decrease of “good” flavors).
2. Strawberry flavored milk becomes more undesirable with time due to the degradation of major milk constituents (increase of “bad” flavors).

Flavor-color interactions play a role in milk liking. Strawberry aroma is paired mainly with pink and, to a lesser extent, red color ($\chi = 420$) (Demattè, 2006). Red colorant used in strawberry flavored milk often is a combination of FD&C Red #3 and FD&C Red#40. Both Red#3 and Red#40 are stable at pH 7, but have only fair oxidative stability (Igoe, 1989). Common milk containers are high density or low density polyethylene (HDPE and LDPE, respectively) and are permeable to gases such as oxygen. Many studies examined various flavor-color interactions with an aroma or taste present (Delwiche 2004, Pangborn 1960, Pangborn and Hanson 1963, Zellnar 1991). Our studies will examine color without added flavor.

3. Strawberry flavor is important in the liking of strawberry milk (e.g. not just sweetness and color).

Chapter II:

The Effect of Vitamins, Sugar, Thickener, and Flavor Additions on Flavor Changes in Stored Low-fat Milk

Introduction

Many studies have been conducted on the sources of common undesirable flavors of milk. For example, it is commonly agreed that good quality, fresh milk has a somewhat bland, slightly salty, and sweet flavor. The common off flavors that are associated with milk include heated, light-induced, lipolyzed, microbial, oxidized, transmitted, and miscellaneous (ADSA Committee on Dairy Products Evaluation, 1979).

• *Heated* flavors are generally the result of free sulfhydryls caused by the breakdown of β-lactoglobulin during pasteurization. These cooked, scorched, and caramelized flavors typically decrease within a few days of the heat treatment (Hutton and Patton, 1952; Shipe et al., 1978).

• *Light-induced* flavors include "activated" flavors caused by the photo-degradation of proteins (such as methionine to methional in the presence of riboflavin) as well as the photo-oxidation of monounsaturated fatty acids from triglycerides (Cadwallader, 1998; Patton, 1954; Allen and Parks, 1975; Leland, 1986, Samuelsson and Harper, 1961; Wishner, 1964).

• *Lipolyzed* flavors are caused by the hydrolysis of triglycerides, which leads to a mixture of volatile short chain free fatty acids (Scanlan et al., 1965; Bills et al., 1969). Lipolyzed flavors are associated with terms such as rancid, butyric, bitter, and goaty (Shipe et al., 1978).

• *Microbial* flavors (such as fruity, malty, and acidic flavors) are often caused by the activity of microorganisms both before and after pasteurization (Bassette et al., 1986).

• *Oxidized* flavor reactions, sometimes catalyzed by copper or iron, are the result of oxidation of polyunsaturated fatty acids on the outside layer of fat globules (Bassette et al., 1986; Leland, 1986). Oxidized flavors can be described as paper, cardboard, metallic, oily, and fishy (Shipe et al., 1978).

• *Transmitted* flavors are those relate to the cow's diet or the cow's milk production physiology (Bassette et al., 1986).

• *Miscellaneous* flavors are sub-classified as any of the following: absorbed taints, astringent, bitter, chalky, chemical, flat, and foreign, or simply a nondescript lack of freshness (Shipe, 1980; Bassette et al., 1986).

In addition to these recognized types of undesirable flavors in milk, various ingredients and flavorings may also have an impact on milk flavor quality. Two common (in fact mandated) ingredients in reduced fat milk that may contribute to off flavors are Vitamins A and D_3. Vitamins A (retinol) and D (calciferol) are fat soluble vitamins. Information was not found on the flavor of vitamins in milk, but fatty carriers may oxidize and contribute off-flavors to the milk. Chapman et al. (1998) found that adding carrageenan and chocolate flavor and color to milk reduced the Vitamin A degradation, but did not reduce light oxidized flavors. Whited et al. (2002) found in milks exposed to fluorescent light that whole milk had less Vitamin A degradation, but developed light oxidized flavor more quickly than did skim milk. Hansen and Metzger (2010) found that adding extra Vitamin D to chocolate milk did not change the flavor or liking of it. Another ingredient in flavored milk that has an impact on flavor perception is sweetener.

Davidson et al. (1999) measured menthone in chewing gum and found that while the quantity of menthone in the breath remained stable over chewing time, the panelists perceived the mint flavor as dissipating until they were given the supporting taste components: sugar/water. Given this data, we will investigate whether sweetener and flavor have the same interaction in flavored milk. Lavin and Lawless (1998) noted that adding vanillin to milk influenced panelists to think the flavored milk was sweeter, creamier, and more likable than unflavored milk.

Antoine and Donawa (1990), Evenson et al. (1988), Pearson and Marth (1990) and Jensen et al. (2001) identified sources of microorganisms causing deterioration (spoilage) of chocolate milk, but did not look at overall milk flavor (especially in non-spoiled milk). In addition, Douglas et al. (2000) found that milk pasteurized with cocoa powder had significantly greater growth of microorganisms after storage for 14 and 21 days than did milk pasteurized without cocoa powder. In accessing the aroma of a strawberry drink, Siegmund et al. (2001) found that the intensity of aroma decreased over time in storage at 27°C. Van Aardt et al. (2001) found that the flavor threshold for an undesirable flavor was significantly greater in chocolate milk than in spring water. This may or may not apply directly to a comparison between flavored and unflavored milk.

Although these studies have identified causes of undesirable flavors, we have not addressed the qualitative or quantitative flavor changes in direct analysis. One way to monitor desirable and undesirable flavor changes is using a sensory panel. A panel trained in descriptive analysis works like a calibrated instrument. In descriptive analysis, a trained panel is presented with samples (in the case of a shelf-life study, the samples have been stored for varying amounts of time) and asked to rate the intensity of flavors, colors, textures, or other sensory attributes using a line scale (Lawless and Heymann, 1999). During training, the panelists are given a representative sampling of products and asked to determine a set of words to describe the differences between these samples. In the related training sessions, panelists are asked to narrow down terms until they have enough to describe the flavors present without having two terms addressing one characteristic (Civille and Lawless, 1986).

Another way to monitor flavor changes is to measure compounds using gas chromatography. Solid Phase Microextraction (SPME) is an extraction technique based on the partitioning of organic compounds between (in this case) a liquid and a vapor phase, and the film/coating on a SPME fiber (Harmon, 1997). An important consideration is the volatility of compounds of interest. Marsili (2000) used SPME to predict the shelf-life of homogenized reduced-fat plain milk and full-fat chocolate milk. The Carboxen-PDMS (polydimethylsiloxane) fiber has been useful in measuring undesirable aromas in plain and chocolate milk (Qian and Burbank, 2007; Marsili, 1999; Marsili, 2000). Vanillin (4-hydroxy-3-methoxy benzaldehyde), an artificial flavor commonly found in vanilla flavored milk, has a boiling point of 285°C and is solid at room temperature (CRC Press Handbook of Chemistry and Physics 1972). This makes determining vanillin content by headspace sampling inconvenient if not impossible. In the last fifteen years, a few studies have involved dipping the SPME fiber directly into the liquid sample (García et al., 1996; García et al., 1997; Lisor and Buszewski, 1999; Urruty et al., 1997). These studies, mostly conducted on wine, did not include a rinsing step, and since the current study involved milk – which contains many proteins, carbohydrates, fats, and thickeners – rinsing may be useful to reduce fiber damage over time.

This first study conducted for our purposes in examining flavored milk used sensory evaluation to look at how flavors change with time in milk as well as GC/SPME to monitor vanillin concentration in vanilla flavored milk. The purpose of this study was to look at how the presence or absence of ingredients changed sensory attribute characteristics and to create a baseline for pleasant and unpleasant flavor ratings for future studies.

Materials and Methods

Materials and Sources

Ingredients

Raw whole cow's milk (Dairy Farmers of America; headquarters in Kansas City, MO); sucrose (commercial mixture); TIC Pretested® Dairy Blend Chocolate 46 which consisted of salt, corn starch, carrageenan (standardized with potassium chloride and dextrose), maltodextrin, and tetrasodium pyrophosphate (TIC Gums, Belcamp, MD); Roche0126 Vitamin A Palmitate (1.0 million IU/g) and Vitamin D3 (100,000 IU/g) (L Hoffman-La Roche Ltd of Basel, Switzerland); Robertet Natural and Artificial Vanilla Flavor Powder Blend NV-31,614 (Robertet Flavors, Inc. Piscataway, NJ); Nestlé unsweetened cocoa powder (Nestlé USA, Inc., Glendale, CA); Hershey's unsweetened cocoa powder (The Hershey Company, Hershey, PA); Robertet Natural Strawberry Flavor WONF with artificial color NV-32,294 (Robertet Flavors, Inc. of Piscataway, NJ)

Equipment

Westphalia MP1254 separator (GEA, Oelde, Germany); Microthermics® UHT/HTST Lab Electric Model 25HV Hybrid (Microthermics® Raleigh, NC); GEA Niro Soavi homogenizer, Type NS2006H, CP-1, S06H-4796 (GEA Copenhagen, Denmark); Microthermics® Clean Fill Hood & Sterile Product Outlet (Microthermics® Raleigh, NC); HP 6890 Series GC (Hewlett Packard, Palo Alto, CA)

Materials

Eight oz. clear PETE containers (Consolidated Plastics Company, Inc. of Twinsburg, OH); Supelco Carboxen™- PDMS SPME fiber (Sigma-Aldrich, St. Louis, MO); SPME liner (78.5mm x 6.3mm OD, 1.5mm ID) (Supelco, Sigma-Aldrich, St. Louis, MO); HP-1 column (Hewlett-Packard Company, Houston, TX); Aerobic Plate Count (APC) Plates (3M™ Petrifilm™, St. Paul, MN); Coliform Count Plates (3M™ Petrifilm™, St. Paul, MN); 2 oz. clear plastic cups (Commercial source); CompuSense software (Guelph, Ontario, Canada); and SAS software (SAS Institute Inc., Cary, NC) were used in this study.

Milk Ingredients

The formulations used in preparing samples are described in Table 1.

Table 1: Modifications made to commercial low-fat milk to create the sample formulations.

	2% fat milk	vitamin fortified [b]	sucrose [c]	thickener [d]	vanilla flavor [e]	cocoa powder [f]	strawberry flavor [g]
Plain Milk (unflavored/ unsweetened milk)	x						
No Vitamins (unflavored/ sweetened milk)	x		x	x			
Control (unflavored/ sweetened milk with vitamins)	x	x	x	x			
Poor Quality [a] (unflavored/ sweetened milk)	x	x	x	x			
Vanilla	x	x	x	x	0.33% by weight		
Chocolate	x	x	x	x	0.08% by weight	x	
Strawberry	x	x	x	x			x

[a] Poor Quality was left at room temperature for an extra 30 min before being pasteurized
[b] Vitamin A Palmitate (1.0 million IU/g) and Vitamin D3 (100,000 IU/g)
[c] sucrose (5% by weight)
[d] TIC Pretested® Dairy Blend Chocolate 46 (0.80% by weight)
[e] Robertet Natural and Artificial Vanilla Flavor Powder Blend NV-31,614
[f] Nestlé unsweetened cocoa powder mixed 1:1 with Hershey's unsweetened cocoa powder (1% by weight)
[g] Robertet Natural Strawberry Flavor WONF with artificial color NV-32,294 (0.97% by weight)

Sample Preparation /Processing

Raw whole cow's milk was separated into cream and skim in the University of Minnesota pilot plant using a Westphalia MP1254 separator. One and a half gallons (12.9 lbs.) of milk was used for each week of each formulation. Both cream and skim were analyzed by the Mojonnier method for fat content (AOAC method 989.05). Raw skim milk and cream were mixed according to Pearson's square to reach a final fat level of 2% by weight. The ingredients for each formulation (as listed in Table 1) were mixed into raw 2% fat milk using a whisk. Each formulation was pasteurized (84°C for 13 sec using a Microthermics® UHT/HTST Lab Electric Model 25HV Hybrid) and homogenized (1500psi) using a GEA Niro Soavi homogenizer, Type NS2006H, CP-1, S06H-4796. Then we filled (using a Microthermics® Clean Fill Hood & Sterile Product Outlet) 8oz. clear PETE containers with the formulations. We then capped the containers and placed them in cardboard boxes to avoid light-induced reactions in a refrigerator at 4-7°C. Milk samples were processed weekly for eight weeks prior to each trial, so all eight weeks of samples could be tasted in the same week.

Due to the large quantity of samples were prepared in three groups. Trial A consisted of Plain Milk, No Vitamins, and Control formulations. Trial B consisted of Poor Quality, Vanilla, and Control formulations. Trial C consisted of Chocolate, Strawberry, and Control formulations. Each group of three milks was tested within the same week. The Control formulation was the same for all trials

Microbial Testing

Milk was tested by Paradigm Diagnostics (St. Paul, MN) for aerobic plate count and coliforms after pasteurization and just prior to presentation to sensory panels using Aerobic Plate Count (APC) Plates and Coliform Count Plates in accordance with AOAC Method 986.33. According to the Pasteurized Milk Ordinance, the APC should be less than 20,000 cfu/ml and the coliform count should be less than 10/ml after pasteurization (US Dept. of Health and Human Services, 1999). Milk was not tasted if the APC exceeded 20,000 cfu/ml, if there were any coliforms present, or if the pH was below 6.0. The pH stipulation was added after the first session of sensory testing.

Descriptive Analysis

A panel of ten people (3 females and 7 males, ages 22-43) recruited from the University of Minnesota Food Science and Nutrition Department developed a list of twenty attributes to describe vanilla, chocolate, and strawberry flavored milk. In their first one and a half hour session, they tasted a variety of flavored milks stored for differing amounts of time and brainstormed words they could use to describe these milks. In their second session, these descriptors were narrowed down and paired with references. The descriptors chosen and their related references are listed in Table 2. In their third session, they scored a practice set of milks on an unlabeled line scale (15 points in length) using their descriptors. During the study, they were presented with various stored milk samples in 2 oz. cups, which were identified by three digit random number codes, and asked to rate the samples on 20 attributes. They were given up to 12 samples at a time, for four sessions in a week, for three non-consecutive weeks. Paper questionnaires were created with CompuSense software.

Table 2: References used for sensory attributes of flavored milks by descriptive analysis panel

Attribute	Definition	References	Intensity (on 15 point universal scale)
<u>Tastes</u>			
Sweet	The taste on the tongue associated with sugars	10% sucrose solution	10
Sour	The taste on the tongue associated with acid	lactic acid solution	None given
Salty	The taste on the tongue associated with sodium chloride	0.2% sodium chloride solution	5
Bitter	The taste on the tongue associated with caffeine	Hershey's unsweetened chocolate	12
Umami	The tastes on the tongue associated with salts of glutamate, aspartate, and some ribonucleotides	MSG	14
<u>Flavors</u>			
Malty	The aromatic associated with toasted malt	Nestle carnation malted milk	7
Butter/diacetyl	The aromatic associated with butter/butter flavored popcorn	Robertet diacetyl – just the smell	15
Cream/fresh dairy	The aromatic with fresh pasteurized cream	Kemps heavy whipping cream	15
		Land O' Lakes Grip 'n Go 2% milk	6
Cooked/caramel	The aromatic associated with burnt or UHT milk and reminiscent of browned sugars	Werther's Original chewy caramels	15
Fruity	The aromatic associated with artificial strawberries	Ethyl butyrate – just the smell	14
Strawberry	The aromatic associated with fresh strawberries	Twizzler's strawberry twists	6
Chocolate	The aromatic associated with milk chocolate	Bug Bites milk chocolate	14
Vanilla	The aromatic associated with vanillin	McCormick imitation vanilla extract – just the smell	15
Green	The aromatic associated with freshly cut grass	Robertet cis-3-hexenol – just the smell	15
Oxidized/cardboardy	The aromatic associated with somewhat oxidized fats and oils reminiscent of wet cardboard	Wet cardboard	2.5
Sulfur/eggy	The aromatic associated with stored hard boiled egg	Hard boiled egg	13
Dirty/smoky/leathery/ earthy/barny	The aromatic associated with smoked meat or cheese	Artificial smoke flavoring – just the smell	15
Chemical/medicinal	The aromatic associated with liquid cough medicine	Luden's wild cherry throat drops	8
Lactones/coconut	The aromatic associated with peach or coconut milk	Gamma decalactone – just the smell	8

Gas Chromatography/Solid Phase Microextraction (GC/SPME)

Vanilla milk samples, in addition to being analyzed by sensory methods, were analyzed using GC/SPME. At the time of testing, three 5 ml aliquots were taken from two bottles of milk (1 from one bottle and 2 from the other bottle) which had been stored for 1 to 8 weeks and poured into separate 20ml vials. Vials containing 7.5mg vanillin/10ml water were interspersed with the weekly vanilla milk samples as references. Each vial was given a code and set on a lab bench for one hour to come to room temperature. A Supelco 75µm Carboxen™- PDMS SPME fiber assembly was used for volatile isolation. The samples were evaluated by GC/SPME after dipping the SPME fiber into the 5ml sample in a 20ml vial for 30 sec at room temperature on the bench-top, being rinsed in water for 15 sec, and then desorbed into the GC inlet (250°C) for 10 min. Sample orders were randomized within each replication using a random number table. The GC inlet had a SPME liner (78.5mm x 6.3mm OD, 1.5mm ID). An HP-1 column was used and the GC oven temperature program was 40°C to 220°C at a rate of 5°C/min. This temperature program was used as we were interested in compounds other than just vanillin in this study. However, only vanillin findings will be discussed in this report.

Statistical Model

All seven recipes were prepared each week for 8 weeks and then evaluated together at one time. Thus, the milks had been stored for varying lengths of time before the tasting week. The model was 3 3x8 factorials. Time of mixing milk was confounded with time stored. Statistical analysis of the sensory studies was calculated using SAS software. The purpose of this study was to define hypothesis #1 and #2. The contrasts of interest were between every milk formulation and the control to determine how each ingredient addition affected the milk flavor. The model included the Judge (the panelist making the ratings – 9 degrees of freedom), the rep (the number of times each judge rated each milk – 1 degree of freedom), the Milk (each treatment – 2 degrees of freedom), the Week (amount of time stored – 5 to 7 degrees of freedom), and the interaction between Milk and Week (7 to 13 degrees of freedom). Samples were considered significantly different if the p-value was less than 0.05. Although these statistics were calculated, they were not discussed.

Results

Microbial Testing

The following tables (Tables 3, 4, and 5) reveal which milks were tested as well as the measured pH and if coliforms were present in microbial testing. All samples had an APC of less than 20,000 cfu/ml.

Table 3: Samples tested in descriptive analysis for sessions 1 and 2 during Trial A. Yes means that the milk was tasted by the panel. The pH of the milk is listed if it was measured.

Week	Plain Milk#1	Plain Milk#2	No Vitamins #1	No Vitamins #2	Control#1	Control#2
1	Yes	Yes, 7.19	Yes	Yes, 7.08	Yes	Yes, 7.06
2	Yes	Yes, 7.17	Yes	Yes, 7.06	Yes	Yes, 7.04
3	Yes	Yes, 7.15	Yes	Yes, 7.05	Yes	Yes, 7.00
4	Yes	Yes, 6.20	Yes	Yes, 6.08	Yes	Yes, 6.11
5	Yes	Yes, 6.52	Yes	No, 5.98	Yes	No, 5.93
6	Yes	Yes, 5.97	Yes	No, 5.75	Yes	No, 5.77
7	No, coliforms	No, coliforms 6.41	Yes	No, 5.65	Yes	No, 5.68
8	yes	Yes, 7.10	Yes	Yes, 7.11	Yes	No, 5.91

Table 4: Samples tested in descriptive analysis for sessions 1 and 2 during Trial B. Yes means that the milk was tasted by the panel. The pH of the milk is listed if it was measured.

Week	Control#1	Control#2	Poor Quality #1	Poor Quality #2	Vanilla #1	Vanilla #2
1	Yes, 7.08	Yes, 6.94	Yes, 7.14	Yes, 6.90	Yes, 7.08	Yes, 6.82
2	Yes, 7.15	Yes, 6.85	Yes, 7.16	Yes, 6.90	Yes, 7.04	Yes, 6.80
3	Yes, 7.15	Yes, 6.96	Yes, 7.17	Yes, 6.85	Yes, 6.95	Yes,6.90
4	Yes, 7.06	Yes, 6.48	Yes, 6.92	Yes, 6.77	Yes, 7.06	Yes, 6.69
5	No, 5.93	No, 5.47	No, 5.88	No, 5.54	Yes, 6.76	Yes, 6.26
6	Yes, 6.01	No, 5.61	Yes, 6.01	No, 5.76	Yes, 6.51	Yes, 6.14
7	No, 5.66	No, 5.36	No, 5.94	Yes, 6.68	Yes, 6.86	Yes, 6.70
8	No, 5.85	No, 5.58	No, 5.82	No, 5.73	Yes, 6.99	No, 5.53

Table 5: Samples tested in descriptive analysis for sessions 1 and 2 during Trial C. Yes means that the milk was tasted by the panel. The pH of the milk is listed if it was measured. In addition, the color of the strawberry milk was noted.

Week	Control#1	Control#2	Chocolate#1	Chocolate#2	Strawberry #1	Strawberry #2
1	Yes, 7.15	Yes, 7.07	Yes, 7.05	Yes, 7.05	Yes, 7.18, pink	Yes, 7.15, pink
2	Yes, 7.11	Yes, 7.14	Yes, 7.02	Yes, 6.73	Yes, 7.10, pink	Yes, 7.10, pink
3	Yes, 7.15	Yes, 6.63	Yes, 6.02	No, 5.79	Yes, 7.08, pink	Yes, 6.28, white
4	Yes, 7.14	Yes, 6.15	Yes, 6.03	No, 5.88	Yes, 7.10, pink	Yes, 6.03, white
5	Yes, 6.83	No, 5.89	No, 5.84	No, 5.85	Yes, 6.05, white	No, 5.94, white
6	No, 5.80	No, 5.74	No, 5.92	No, 5.86	No, 5.80, white	No, 5.78, white
7	No, 5.90	No, 5.85	No, 5.82	No, 5.73	Yes, 6.27, white	No, 5.82, white
8	No, coliforms, 6.01	No, coliforms, 5.49	No, coliforms, 5.58	No, coliforms, 5.52	No, coliforms, 6.05, white	No, coliforms, 5.98, white

Milk Preparation

In this study we chose to prepare new milks each week over 8 weeks and then test all of the milks in each formulation at one time. We chose to do it this way, so that all lengths of storage for each formulation could be compared to each other at one time. Interestingly, differences in milks or milk processing resulted in each milk sample having a unique shelf-life, resulting in early spoilage of some milk samples. However, in retrospect we still feel that the benefit of having all samples ready for sensory analysis at the same time outweighed this problem.

Descriptive Analysis

Trial A Comparison of Attributes of Plain Milk, No Vitamins, and Control

Mandatory ingredient additions to milk were assessed to determine if they resulted in perceived flavor differences. The three samples in trial A were plain unflavored milk, milk with mandatory vitamins, and milk with mandatory vitamins and the sugar and thickener necessary for the base of flavored milk. Data on attribute changes (with data for weeks 1 through 8 averaged together for each milk recipe) were compared in order to determine how the addition of sugar and thickener, vitamins, and milk each influence milk flavor. Attribute changes for each milk recipe were tested to determine how the flavor attributes changed in stored milk. Table 6 shows the General Least Squares Means analysis of the milks for Trial A. The mean ratings on attributes for Plain Milk, No Vitamins, and Control are compared in Table 7 to determine how each change in ingredient affected attribute ratings.

Table 6: Significance of various factors on attribute ratings in flavored milk in Trial A. The factors are listed with their related degrees of freedom, F test result, and p-value. Type III SS was used due to uneven groupings.

Attribute	Judge	Rep	Milk	Week	Milk*week
Sweet	9, 9.30, <0.0001	1, 0.58, 0.4455	2, 209.76, <0.0001	7, 15.22, <0.0001	13, 3.68, <0.0001
Sour	9, 10.35, <0.0001	1, 0.03, 0.8632	2, 0.48, 0.6197	7, 51.10, <0.0001	13, 0.75, 0.7152
Salty	9, 14.61, <0.0001	1, 4.51, 0.0343	2, 65.04, <0.0001	7, 2.59, 0.0128	13, 1.48, 0.1234
Bitter	9, 14.45, <0.0001	1, 0.67, 0.4152	2, 12.35, <0.0001	7, 18.34, <0.0001	13, 1.71, 0.0571
Umami	9, 39.91, <0.0001	1, 1.29, 0.2574	2, 2.03, 0.1332	7, 0.27, 0.9641	13, 0.66, 0.8052
Malty	9, 32.69, <0.0001	1, 1.71, 0.1919	2, 26.76, <0.0001	7, 3.16, 0.0029	13, 2.07, 0.0154
Butter	9, 13.50, <0.0001	1, 9.78, 0.0019	2, 6.39, 0.0019	7, 1.14, 0.3393	13, 0.87, 0.5863
Cream	9, 15.06, <0.0001	1, 0.98, 0.3238	2, 0.04, 0.9562	7, 20.45, <0.0001	13, 1.27, 0.2288
Cooked	9, 20.91, <0.0001	1, 0.01, 0.9203	2, 34.52, <0.0001	7, 3.70, 0.0007	13, 2.10, 0.0136
Fruity	9, 46.86, <0.0001	1, 1.51, 0.2206	2, 21.13, <0.0001	7, 9.94, <0.0001	13, 2.32, 0.0057
Strawberry	9, 8.92, <0.0001	1, 0.79, 0.3761	2, 17.76, <0.0001	7, 8.27, <0.0001	13, 5.42, <0.0001
Chocolate	9, 311.46, <0.0001	1, 0.16, 0.6900	2, 0.91, 0.4036	7, 0.66, 0.7037	13, 0.62, 0.8378
Vanilla	9, 11.88, <0.0001	1, 0.89, 0.3465	2, 18.95, <0.0001	7, 3.01, 0.0044	13, 1.49, 0.1191
Green	9, 8.50, <0.0001	1, 1.56, 0.2122	2, 4.15, 0.0165	7, 8.59, <0.0001	13, 1.19, 0.2849
Oxidized	9, 17.22, <0.0001	1, 0.20, 0.6559	2, 2.77, 0.0639	7, 12.40, <0.0001	13, 1.05, 0.3993
Sulfur	9, 15.35, <0.0001	1, 0.21, 0.6469	2, 6.87, 0.0012	7, 7.92, <0.0001	13, 2.56, 0.0022
Dirty	9, 13.37, <0.0001	1, 0.15, 0.7020	2, 1.64, 0.1962	7, 12.17, <0.0001	13, 0.86, 0.5990
Chemical	9, 9.37, <0.0001	1, 1.66, 0.1986	2, 3.60, 0.0284	7, 12.05, <0.0001	13, 1.60, 0.0837
Lactones	9, 22.52, <0.0001	1, 6.39, 0.0119	2, 5.55, 0.0042	7, 2.12, 0.0406	13, 1.13, 0.3337
Other off	8, 13.25, <0.0001	1, 2.17, 0.1419	2, 4.06, 0.0182	7, 10.36, <0.0001	13, 1.50, 0.1167

Table 7: The effect of adding sugar, thickener, and vitamins to low-fat milk

Attribute	Plain Milk (Weeks 1-6, 8)	No Vitamins (Weeks 1-8)	Control (Weeks 1-8)
Sweet	2.1[a]	7.3[b]	7.7[b]
Salty	1.5[a]	3.9[b]	3.9[b]
Bitter	3.0[b]	1.7[a]	1.7[a]
Malty	1.3[a]	2.4[b]	2.7[b]
Butter/ diacetyl	1.9[a]	2.9[b]	2.8[b]
Cooked/ caramel	0.9[a]	2.5[b]	2.6[b]
Fruity	1.5[a]	2.1[b]	2.5[b]
Strawberry	0.5[a]	0.6[a]	1.0[b]
Vanilla	0.5[a]	1.4[b]	1.4[b]
Green	1.5[b]	1.0[a]	1.3[a,b]
Oxidized/ cardboardy	3.8[b]	3.4[a,b]	2.9[a]
Sulfur/ eggy	1.2[a]	1.8[b]	1.2[a]
Lactones/ coconut	1.5[a]	2.3[b]	2.2[b]
Other off flavors	2.6[b]	2.1[a,b]	1.7[a]

Means within each attribute with different letters are significantly different than each other at $\alpha=0.05$ using Student-Newman-Keuls Test. Ratings are on an unlabeled line scale (15 points in length). The comparisons are made between milk formulations with data for weeks 1 through 8 averaged together for each milk formulation. Each value is an average of 2 ratings each by ten panelists for each week of storage unless noted in Table 3.

Trial B Comparison of Attributes of Poor Quality, Vanilla, and Control

The influences of microbial growth and of adding vanilla flavor on the sensory perception of milk were considered in this trial. All of the milks in trial B contained sugar, thickener, and vitamins, but one had been left at room temperature for a half hour when raw, and one milk had vanilla flavor added. Data on attribute changes (with data for weeks 1 through 8 averaged together for each milk recipe) were compared in order to determine how the possible growth of microorganisms and the addition of vanilla flavor influence milk flavor. Attribute changes for each milk recipe were evaluated to determine how the flavor attributes changed in stored milk. Table 8 shows the General Least Squares Means analysis of the milks for Trial B. The mean ratings on attributes for Poor Quality, Vanilla, and Control are compared in Table 9 to determine how each change in process or ingredient affected attribute ratings.

Table 8: Significance of various factors on attribute ratings in flavored milk in Trial B. The factors are listed with their related degrees of freedom, F test result, and p-value. Type III SS was used due to uneven groupings.

Attribute	Judge	Rep	Milk	Week	Milk*week
Sweet	9, 71.53, <0.0001	1, 6.53, 0.0111	2, 1.49, 0.2270	7, 7.71, <0.0001	9, 3.71, 0.0002
Sour	9, 11.63, <0.0001	1, 0.00, 0.9901	2, 4.12, 0.0171	7, 35.37, <0.0001	9, 2.34, 0.0147
Salty	9, 28.51, <0.0001	1, 0.32, 0.5702	2, 1.70, 0.1850	7, 1.45, 0.1829	9, 1.58, 0.1211
Bitter	9, 9.99, <0.0001	1, 3.19, 0.0750	2, 0.40, 0.6703	7, 6.96, <0.0001	9, 3.15, 0.0012
Umami	9, 37.60, <0.0001	1, 0.26, 0.6114	2, 0.00, 0.9956	7, 0.59, 0.7616	9, 1.36, 0.2054
Malty	9, 24.03, <0.0001	1, 4.93, 0.0272	2, 2.06, 0.1292	7, 0.62, 0.7356	9, 1.68, 0.0940
Butter	9, 13.24, <0.0001	1, 0.76, 0.3834	2, 0.74, 0.4789	7, 0.25, 0.9708	9, 1.99, 0.0396
Cream	9, 28.97, <0.0001	1, 5.33, 0.0217	2, 1.51, 0.2231	7, 4.30, 0.0001	9, 1.83, 0.0624
Cooked	9, 18.39, <0.0001	1, 9.76, 0.0019	2, 9.14, 0.0001	7, 2.15, 0.0388	9, 1.28, 0.2488
Fruity	9, 14.02, <0.0001	1, 2.19, 0.1402	2, 4.71, 0.0097	7, 0.98, 0.4454	9, 0.55, 0.8362
Strawberry	9, 4.04, <0.0001	1, 2.80, 0.0952	2, 3.99, 0.0195	7, 0.37, 0.9201	9, 0.93, 0.4968
Chocolate	9, 11.03, <0.0001	1, 0.31, 0.5798	2, 2.46, 0.0875	7, 1.31, 0.2468	9, 1.39, 0.1920
Vanilla	9, 21.25, <0.0001	1, 7.93, 0.0052	2, 39.70, <0.0001	7, 6.00, <0.0001	9, 2.82, 0.0034
Green	9, 3.74, 0.0002	1, 4.62, 0.0324	2, 0.21, 0.8143	7, 2.07, 0.0461	9, 1.40, 0.1855
Oxidized	9, 8.24, <0.001	1, 2.10, 0.1479	2, 0.03, 0.9681	7, 12.44, <0.0001	9, 2.01, 0.0376
Sulfur	9, 35.20, <0.0001	1, 1.77, 0.1841	2, 4.61, 0.0106	7, 6.10, <0.0001	9, 3.20, 0.0010
Dirty	9, 10.34, <0.0001	1, 0.02, 0.8918	2, 1.86, 0.1577	7, 16.76, <0.0001	9, 3.73, 0.0002
Chemical	9, 7.06, <0.0001	1, 3.28, 0.0710	2, 11.43, <0.0001	7, 6.68, <0.0001	9, 2.44, 0.0107
Lactones	9, 20.37, <0.0001	1, 2.51, 0.1141	2, 2.66, 0.0719	7, 4.48, <0.0001	9, 2.27, 0.0178
Other off	8, 5.79, <0.0001	1, 8.06, 0.0049	2, 7.39, 0.0007	7, 8.07, <0.0001	9, 3.20, 0.0011

Table 9: The effect of adding vanilla flavor to sweetened, thickened milk

Attribute	Poor Quality (Weeks 1-4,6-7)	Vanilla (Weeks 1-8)	Control (Weeks 1-4,6)
Cream/ fresh dairy	$3.2^{a,b}$	2.7^{a}	3.4^{b}
Cooked/ caramel	2.4^{a}	3.2^{b}	2.3^{a}
Fruity	1.6^{a}	2.5^{b}	2.1^{b}
Strawberry	0.4^{a}	0.7^{b}	0.5^{a}
Vanilla	2.2^{a}	4.4^{b}	2.0^{a}
Dirty/smoky	1.9^{a}	3.0^{b}	1.9^{a}
Chemical/ medicinal	1.0^{a}	2.6^{b}	1.2^{a}
Other off flavors	1.1^{a}	2.4^{b}	0.9^{a}

Means within each attribute with different letters are significantly different than each other at α=0.05 using Student-Newman-Keuls Test. Ratings are on an unlabeled line scale (15 points in length). The comparisons are made between milk formulations with data for weeks 1 through 8 averaged together for each milk formulation. Each value is an average of 2 ratings each by ten panelists for each week of storage unless noted in Table 4.

Trial C Comparison of Attributes of Chocolate, Strawberry, and Control

How the addition of chocolate and strawberry milk flavors changed the flavor perception of milk was considered for this third trial. Again, all of the milks in trial C contained sugar, thickener, and vitamins, but one had added cocoa powder and vanillin and another had added strawberry flavor and color. Data on attribute changes (with data for weeks 1 through 8 averaged together for each milk recipe) were compared in order to determine how the addition of strawberry and chocolate flavors influence milk flavor. Attribute changes for each milk recipe were measured to determine how the flavor attributes changed in stored milk. Table 10 shows the General Least Squares Means analysis of the milks for Trial C. The mean ratings on attributes for Chocolate, Strawberry, and Control are compared in Table 11 to determine how each change in ingredient affected attribute ratings.

Table 10: Significance of various factors on attribute ratings in flavored milk in Trial C. The factors are listed with their related degrees of freedom, F test result, and p-value. Type III SS was used due to uneven groupings.

Attribute	Judge	Rep	Milk	Week	Milk*week
Sweet	9, 25.72, <0.0001	1, 1.12, 0.2920	2, 18.73, <0.0001	5, 3.01, 0.0120	7, 2.63, 0.0124
Sour	9, 11.39, <0.0001	1, 1.32, 0.2525	2, 9.11, 0.0002	5, 28.67, <0.0001	7, 2.76, 0.0091
Salty	9, 26.12, <0.0001	1, 0.02, 0.8970	2, 1.82, 0.1636	5, 1.93, 0.0911	7, 0.90, 0.5092
Bitter	9, 18.57, <0.0001	1, 7.64, 0.0062	2, 43.07, <0.0001	5, 1.55, 0.1753	7, 1.00, 0.4321
Umami	9, 34.93, <0.0001	1, 0.38, 0.5392	2, 1.86, 0.1581	5, 1.75, 0.1233	7, 1.24, 0.2795
Malty	9, 32.41, <0.0001	1, 0.18, 0.6725	2, 7.58, 0.0007	5, 0.36, 0.8780	7, 0.63, 0.7344
Butter	9, 14.63, <0.0001	1, 0.02, 0.8784	2, 15.67, <0.0001	5, 0.51, 0.7719	7, 1.06, 0.3936
Cream	9, 22.16, <0.0001	1, 0.01, 0.9158	2, 13.43, <0.0001	5, 3.30, 0.0067	7, 3.28, 0.0025
Cooked	9, 31.03, <0.0001	1, 4.50, 0.0349	2, 1.83, 0.1635	5, 0.26, 0.9335	7, 0.55, 0.7978
Fruity	9, 26.15, <0.0001	1, 0.12, 0.7320	2, 64.29, <0.0001	5, 2.29, 0.0466	7, 0.83, 0.5627
Strawberry	9, 2.34, 0.0153	1, 0.52, 0.4717	2, 130.28, <0.0001	5, 3.81, 0.0025	7, 4.62, <0.0001
Chocolate	9, 1.71, 0.0868	1, 0.36, 0.5492	2, 494.17, <0.0001	5, 0.04, 0.9993	7, 0.07, 0.9994
Vanilla	9, 7.78, <0.0001	1, 2.72, 0.1007	2, 8.03, 0.0004	5, 1.15, 0.3348	7, 0.37, 0.9209
Green	9, 13.40, <0.0001	1, 0.15, 0.7014	2, 11.90, <0.0001	5, 0.99, 0.4248	7, 0.54, 0.8025
Oxidized	9, 8.97, <0.0001	1, 4.21, 0.0412	2, 7.18, 0.0009	5, 4.74, 0.0004	7, 0.41, 0.8954
Sulfur	9, 12.54, <0.0001	1, 1.02, 0.3140	2, 4.65, 0.0105	5, 1.27, 0.2790	7, 0.96, 0.4601
Dirty	9, 10.28, <0.0001	1, 3.45, 0.0647	2, 5.18, 0.0063	5, 6.26, <0.0001	7, 1.06, 0.3880
Chemical	9, 13.68, <0.0001	1, 0.15, 0.6959	2, 18.19, <0.0001	5, 0.87, 0.5023	7, 0.72, 0.6576
Lactones	9, 22.15, <0.0001	1, 1.10, 0.2945	2, 10.16, <0.0001	5, 0.73, 0.6039	7, 0.69, 0.6777
Other off	8, 16.03, <0.0001	1, 0.02, 0.8875	2, 8.28, 0.0003	5, 10.98, <0.0001	7, 6.51, <0.0001

Table 11: The effect of adding chocolate or strawberry flavor to sweetened, thickened milk

Attribute	Chocolate (Weeks 1-4)	Strawberry (Weeks 1-5,7)	Control (Weeks 1-5)
Sweet	5.5[a]	7.3[b]	7.2[b]
Bitter	3.7[c]	1.9[b]	1.1[a]
Malty	2.6[b]	1.5[a]	2.3[b]
Butter/ diacetyl	0.9[a]	1.3[b]	2.0[c]
Cream/ fresh dairy	1.6[a]	1.9[a]	2.8[b]
Fruity	1.4[a]	5.4[c]	2.5[b]
Strawberry	0.4[a]	6.3[b]	0.8[a]
Chocolate	8.8[b]	0.4[a]	0.4[a]
Vanilla	0.8[a]	1.7[b]	2.2[b]
Green	0.7[a]	2.3[b]	1.1[a]
Oxidized/ cardboardy	1.7[a]	1.5[a]	2.5[b]
Sulfur/ eggy	0.7[a]	0.8[a]	1.1[b]
Chemical/ medicinal	1.4[a]	3.7[b]	1.7[a]
Lactones/ coconut	0.8[a]	1.4[b]	2.0[c]

Means within each attribute with different letters are significantly different than each other at α=0.05 using Student-Newman-Keuls Test. Ratings are on an unlabeled line scale (15 points in length). The comparisons are made between milk formulations with data for weeks 1 through 8 averaged together for each milk formulation. Each value is an average of 2 ratings each by ten panelists for each week of storage except as noted in Table 5.

The flavor changes in Tables 7, 9, and 11 are summarized in Table 12.

Table 12: The effect of adding ingredients to low-fat milk on discernment of flavor attributes.

Attribute	Trial A	Trial B	Trial C
Sweet	G +		C -
Sour			
Salty	G +		
Bitter	G -		
Umami			
Malty	G +		S -
Butter/diacetyl	G +		S - , C --
Cream/fresh dairy		V -	S -, C -
Cooked/caramel	G +	V +	
Fruity	G +		S +, C -
Strawberry	B +	V +	S +
Chocolate			C +
Vanilla	G +	V +	C -
Green			S +
Oxidized/ cardboardy			S -, C -
Sulfur/eggy			S -, C -
Dirty/smoky		V +	
Chemical/medicinal		V +	S +
Lactones/coconut	G +		S -, C --
Other off flavors		V +	

+ denotes increase in mean rating of attribute when ingredient was added. – denotes decrease in mean rating of attribute when ingredient was added. G = sugar/gums added; B = vitamins added; V = vanilla flavoring added; C = chocolate flavoring added; S = strawberry flavoring added

The previous tables compared formulations to each other. The changes in each formulation of milk over time are listed in Table 13.

Table 13: The effect of time on flavor changes in different formulations of milk.*

Attribute	Plain Milk	No Vitamins	Control – trial A	Control – trial B	Control – trial C	Poor Quality	Vanilla	Chocolate	Strawberry
Sweet		decrease	decrease				decrease		varied
Sour	increase	increase	increase	increase	increase	varied	increase	increase	varied
Bitter	increase	increase	increase	increase	increase	varied			
Cream/ fresh dairy	decrease	decrease	decrease		decrease		decrease		
Fruity			increase						
Strawberry			increase						decrease
Vanilla			decrease				decrease		
Green	increase		increase	increase	increase				
Oxidized/ cardboardy	increase	increase	increase		increase	varied	increase		increase
Sulfur/ eggy		increase				varied			
Dirty/ smoky	increase	increase	increase	increase	increase	varied	increase		increase
Chemical/ medicinal	increase	increase	increase			varied	increase		
Lactones/ coconut				varied					
Other off flavors	increase	increase	increase		increase	varied	increase		

*If changes were significant at $p<0.05$, the direction of change is listed.

Gas Chromatography/Solid Phase Microextraction (GC/SPME)

Concentrations of vanillin in milks were calculated based on the analysis of a 7.5mg vanillin/10 ml water solution. Other than Week 6, the vanillin in the vanilla flavored milk remained within 0.2 mg/10 ml of 3.5 mg vanillin/10 ml solution (milk) over the eight weeks storage time. Week 6 (at 1.6 mg/10 ml) is an outlier. Week 6 milk also varied in the poor quality treatment milk, having much higher ratings of unpleasant attributes than in previous or later weeks. Milk was tested for absence of coliforms, but week 6 may have had an unusually high number of microorganisms, which, in turn, fed in some manner on the vanillin.

Discussion

The pH of milk varied widely even within samples of the same treatment and storage time. Further study conducted using thermocouples suggested that samples stored near the edges of the cardboard boxes cooled more quickly than those stored in the center of those same boxes. In the future, something should be done to control the cooling of the samples. One more interesting occurrence was that the pH of Vanilla flavored milk decreased slower than in the other milks.

For the purposes of defining hypotheses, the sensory attributes that are affected by the milk itself are sour, bitter, oxidized/cardboardy, dirty/smoky, and other off flavors can be generally categorized as unpleasant flavors. These attributes increased with time in at least three out of five of the unflavored milks and had an overall mean rating of at least one in strawberry flavored milk. Green, fruity, and chemical/medicinal notes were most prominent in the Strawberry flavored milk, but changed most in unflavored milk, so are therefore harder to categorize. Therefore, changes in sour, bitter, oxidized/cardboardy, dirty/smoky, and other off flavors will be used to test the following hypothesis: strawberry flavored milk becomes more undesirable with time due to the degradation of major milk constituents (increase of “bad” flavors).

The attributes affected by the addition of sweetener and flavor are most prominently sweet, strawberry, chocolate, and vanilla. These attributes can generally be categorized as pleasant flavors. Cream/fresh dairy notes were masked by the addition of flavoring, so they would be hard to measure in flavored milk. Therefore, changes in sweet, strawberry (for strawberry flavored milk), chocolate (for chocolate flavored milk) and vanilla (for vanilla flavored milk) will be used to test the following hypothesis: strawberry flavored milk becomes more undesirable with time due to the degradation of strawberry flavor (decrease of “good” flavors).

This study examined how the addition of various ingredients changed the flavor of milk. To do this, a sensory panel was trained and one ingredient at a time was changed in the milk formulations. Adding sweetener and thickener increased sweet, salty, malty, butter/diacetyl, cooked/caramel, vanilla, and lactones/coconut notes and decreased bitter notes. Adding vitamins did not result in changes in flavor perception. Leaving milk at room temperature for an extra 30 min did not change flavor perception either. We can conclude that 30 min is a safety margin. Adding strawberry and chocolate flavor masked butter/diacetyl, oxidized/cardboardy, sulfur/eggy, and lactones/coconut attributes. Many individual differences between the control and vanilla, strawberry, and chocolate were directly related to the complexity of the flavor itself. Interestingly, the perception of the flavor of vanilla in vanilla flavored milk decreased with time although the concentration of vanillin in the milk remained constant. Also we found that adding any flavor decreased cream/fresh dairy notes and that the addition of both sweetener and thickener affected many flavor attributes. Finally, we found that strawberry and chocolate milks did not decrease in sweet intensity over time.

Conclusions

As stated in the discussion, parameters for hypotheses #1 and #2 were defined as follows.

• Hypothesis #1: Decreases in sweet taste and strawberry aroma will be used to denote degradation of strawberry flavor.

• Hypothesis #2: Increases in sour, bitter, oxidized/cardboardy, dirty/smoky, and other off flavors will be used to denote increases in undesirable flavors with time.

Future Research

Storage stability issues related to putting our milk bottles directly into cardboard boxes in the refrigerator after pasteurization arose. Instead of doing this in the future, milk bottles were put on ice immediately after being filled with pasteurized milk to insure more rapid and consistent cooling. Pasteurization temperature was also be increased from 84°C to 89°C due to the addition of solids.

Chapter III:

The Effect of Added Flavor, Colors, and Time on Flavor Attribute Changes in Low-fat Milk

Introduction

Many studies have been done on the flavor of unflavored milk, but few studies have been conducted on flavored milk. We will now review what has been written about the effects additional flavors have on milk. Please refer to the milk discussion in the introduction of Chapter 2.

Strohman et al. (Chapter 2) found in pasteurizing flavored milk at 84°C for 13 sec and storing the bottles of milk immediately in cardboard boxes in a refrigerator (4-7°C), that a great deal of variability of pH between milk samples was present, suggesting microbial growth. Strohman suggested raising the pasteurization temperature and cooling bottles of pasteurized milk on ice before transferring it to the refrigerator to reduce this problem.

Although microbial growth affects flavor, it is not the only thing to consider. Scanlan et al. (1965) and Golaszewski et al. (1998) identified (Z)-3-hexenal, 4-hydroxy-2,5-dimethyl-3(2H) furanone, 2,5-dimethyl-4-methoxy-3(2H) furanone, and methyl butanoate as key aroma compounds in strawberry juice, but this was in fruit juice rather than in flavored milk. Siegmund et al. (2001) found that the intensity of aroma of a strawberry drink (strawberry pulp, sugar, and concentrated juice in a water base) decreased over time in storage at 27°C. Van Aardt et al. (2001) found that the flavor threshold for an undesirable flavor was significantly greater in chocolate milk than in spring water. Strohman et al. (Chapter 2) found that adding strawberry or chocolate flavors to milk masked some undesirable attributes such as oxidized and sulfur aromas. They also found that even though panelists perceived vanilla flavor as decreasing with time in vanilla flavored milk, the actual vanillin concentration of the milk remained constant. Lavin and Lawless (1998) noted that adding vanillin to milk influenced panelists to think the flavored milk was sweeter, creamier, and more likable than unflavored milk. They also found that changing the color intensity in fruit beverages led panelists to rate the beverages as having different sweet taste intensities. For example, adults thought that dark red strawberry beverage was significantly sweeter than light red strawberry beverage, while light green key lime beverage was significantly sweeter than dark green key lime beverage).

Our hypotheses are that desirable flavor attributes (sweet, strawberry, chocolate and vanilla) will decrease and that undesirable flavor attributes (sour, bitter, oxidized/cardboardy, dirty/smoky and other off flavors) will increase in flavored milk over its useable shelf-life. In addition, we hypothesize that strawberry colored (but unflavored) milk will be rated as having more strawberry flavor than unflavored, uncolored milk, but less strawberry flavor than strawberry colored and flavored milk. We further hypothesize that milk with flavor added just prior to tasting will have more strawberry flavor than milk with flavor added prior to pasteurization.

Materials and Methods

Materials and Sources

Ingredients

The ingredients used in this study were the same as those used in Chapter 2 except a commercial mixture of Vitamins A and D_3 was used instead of the Roche0126 vitamins listed in Chapter 2 and Robertet's University-of-Minnesota- Control PW-2-82-3 (NV-32,294 without flavor components, i.e. color only) (Robertet Flavors, Inc. Piscataway, NJ) was added.

Equipment

The equipment used in the study were the same as those used in Chapter 2 except a Lightenin Mixer, Rotation, Model C4 (Mixing Equipment Co., Inc.) was used for mixing and an HP 6890 Series GC was not used.

Materials

Aerobic Plate Count (APC) Plates and Coliform Count Plates (3M™ Petrifilm™, St. Paul, MN); 16oz. opaque HDPE containers (commercial source); 2 oz. clear plastic cups (Commercial source)

Software

SIMS2000 software (Sensory Information Management System, Morristown, NJ); SAS software (SAS Institute Inc., Cary, NC)

Milk Ingredients

The description of the composition of milk formulations can be found in Table 14. A variety of milk formulations were observed. Unflavored/ unsweetened milk (Unf-Uns) was chosen as a representation of regularly purchased reduced fat milk. Vanilla flavored milk (Van), Chocolate flavored milk (Choc), and Strawberry flavored milk (StFlv) were chosen to assess popular flavored milk flavors. Strawberry colored, unflavored milk (StCol) was chosen to determine how much influence color has on perceived flavor. Milk with strawberry flavoring added just prior to testing (StFlvPtt) was chosen to consider how or if pasteurization and storage changes strawberry flavor.

Table 14: Modifications made to 2% fat milk to create the sample formulations.

	vitamins A & D3	sucrose [a]	gum blend [b]	vanilla flavor [c]	cocoa powder [d]	artificial color [e]	strawberry flavor & color [f]	flavor added post-pasteurization
Unflavored/ unsweetened milk (Unf-Uns)	x							
Vanilla flavored milk (Van)	x	x	x	(0.33% by weight)				
Chocolate flavored milk (Choc)	x	x	x	(0.08% by weight)	x			
Strawberry colored, unflavored milk (StCol)	x	x	x			x		
Strawberry flavored milk (StFlv)	x	x	x				x	
Milk with strawberry flavoring added just prior to testing (StFlvPtt)	x	x	x				x	x

[a] sucrose (5% by weight)
[b] TIC Pretested® Dairy Blend Chocolate 46 (0.80% by weight)
[c] Natural and Artificial Vanilla Flavor Powder Blend NV-31,614
[d] Mixture of unsweetened Nestlé unsweetened cocoa powder mixed 1:1 with Hershey's unsweetened cocoa powder (1% by weight)
[e] artificial color NV-32,294 (0.97% by weight)
[f] Natural Strawberry Flavor WONF with artificial color NV-32,294 (0.97% by weight)

Sample Preparation/Processing

Raw, whole fat, cow's milk was obtained from Dairy Farmers of America and was separated into cream and skim milk in the University of Minnesota pilot plant using a Westphalia MP1254 separator. Fifteen gallons (about 129 lbs.) of milk was used for each formulation. Both cream and skim were analyzed by IR for fat content by DQCI Services (Mounds View, MN). Raw skim milk and cream were mixed according to Pearson's square to reach a final fat level of 2% by weight. Unf-Uns contained only 2% milk and the commercial vitamin mixture. Additional ingredients and formulations are listed in Table 9. Formulations were mixed using a Lightenin Mixer and then pasteurized (89°C for 13 sec using a Microthermics® UHT/HTST Lab Electric Model 25HV Hybrid) and homogenized (1500psi) using a GEA Niro Soavi homogenizer, Type NS2006H, CP-1, S06H-4796. Then we filled (using a Microthermics® Clean Fill Hood & Sterile Product Outlet) 16oz. opaque HDPE containers (sterilized with 200ppm hypochlorite solution and rinsed with distilled, autoclaved water) with the formulations. After capping the containers, we placed them in an ice bath to facilitate rapid cooling the milk. Each batch of flavored milk was stored in a refrigerator (4-7°C) until used in analysis. Milk was processed all in one day in large batches and analyzed each week until it failed microbiological tests.

Microbial Testing

Microbial testing parameters are listed in Chapter 2. The only differences in this study were that milk was not tasted if the APC exceeded 1,000 cfu/ml (rather than 20,000 cfu/ml in Chapter 2) and we did not use pH testing.

Descriptive Analysis

A panel of ten students developed a list of descriptors for describing flavored milk. This process is discussed in Chapter 2. The only difference in references from Chapter 2 is that a scale of diluted citric acid mixtures was used as a sour reference rather than lactic acid (see Table 15). A second group of panelists, ten University of Minnesota students (6 females and 4 males, ages 23 to 45 recruited by departmental email announcements), was presented with various stored milk samples in 2 oz. cups (which were identified by three digit random number codes) and asked to rate the samples on the 20 attributes developed by the previous panel. The training for this second group consisted of a one and a half-hour session acquainting panelists with references for the 20 attributes and a practice session using an unlabeled line scale (with a length of 15 points) on a subset of milk samples. The samples for the study itself were presented weekly in duplicate to this second panel. Note that the samples did not have the same code number as their duplicates. Rating questionnaires were created with SIMS2000 software. Statistical analysis of the sensory studies was calculated using SAS software. Sensory testing was conducted by the Sensory Center at the University of Minnesota (St. Paul, MN).

Table 15: References used for sour attributes of flavored milks by descriptive analysis panel

Attribute	Definition	References	Intensity (on 15 point universal scale)
Sour	The taste on the tongue associated with citric acids	0.050% citric acid solution	2
		0.057% citric acid solution	3
		0.066% citric acid solution	4
		0.075% citric acid solution	5
		0.087% citric acid solution	6
		0.099% citric acid solution	7
		0.114% citric acid solution	8
		0.131% citric acid solution	9
		0.150% citric acid solution	10

Statistical Design

This study used a used a 6 x 6 factorial with repeated measures as it contained six milk formulations tested over six weeks. All milks were processed on the same day and then tested each week. Contrasts of interest included comparing strawberry colored milk and strawberry flavored milk for strawberry flavor as well as comparing flavored milk and unflavored milk for undesirable and desirable attributes. The model included the Judge (the panelist making the ratings – 9 degrees of freedom), the rep (the number of times each judge rated each milk – 1 degree of freedom), the Milk (each treatment – 5 degrees of freedom), the Week (amount of time stored – 7 degrees of freedom), and the interaction between Milk and Week (25 degrees of freedom). Samples were considered significantly different if the p-value was less than 0.05. Although these statistics were calculated, they were not discussed.

Results and Discussion

Milk Preparation

In this study, we chose to increase the temperature of pasteurization from 84°C to 89°C and to put the bottles of milk on ice to cool after pasteurization (rather than placing the bottles directly into a cardboard box in the refrigerator). This was due to our previous study having a great variability in shelf-life. We also processed all of the milk (albeit in separate batches) all in one day rather than each week to decrease the variability in the base milk. Even though we tightened the microbial parameters, we were able to have all milk formulations tasted for at least six weeks, which was an improvement. In the previous study (Chapter 2), most milk formulations failed to pass microbial limits after four weeks of storage. The exceptions to this were milk with nothing added (e.g. no sugar, thickener, vitamins, or flavor added) and vanilla flavored milk.

Descriptive Analysis

Table 16 lists the relevant statistics calculated for this study. Interactions were graphed, but they will not be shown or discussed in this paper.

Table 16: Significance of various factors on attribute ratings in flavored milk. The factors are listed with their related degrees of freedom, F test result, and p-value. Type III SS was used due to uneven groupings.

Attribute	Judge	Rep	Milk	Week	milk*week
Sweet	9, 87.84, <0.0001	1, 7.98, 0.0049	5, 138.35, <0.0001	7, 5.88, <0.000q	25, 0.88, 0.6358
Sour	9, 9.67, <0.0001	1, 0.04, 0.8516	5, 31.38, <0.0001	7, 20.28, <0.0001	25, 10.04, <0.0001
Salty	9, 15.76, <0.0001	1, 5.14, 0.0237	5, 29.09, <0.0001	7, 1.13, 0.3443	25, 0.56, 0.9616
Bitter	9, 6.48, <0.0001	1, 2.00, 0.1577	5, 110.23, <0.0001	7, 0.32, 0.9431	25, 0.51, 0.9773
Umami	9, 33.32, <0.0001	1, 0.21, 0.6483	5, 2.91, 0.0132	7, 1.31, 0.2435	25, 0.66, 0.8974
Malty	9, 34.42, <0.0001	1, 0.69, 0.4058	5, 33.76, <0.0001	7, 4.93, <0.0001	25, 0.43, 0.9936
Butter	9, 49.16, <0.0001	1, 0.05, 0.8309	5, 12.47, <0.0001	7, 1.98, 0.0560	25, 1.18, 0.2517
Cream	9, 60.79, <0.0001	1, 2.08, 0.1498	5, 31.01, <0.0001	7, 13.68, <0.0001	25, 1.47, 0.0654
Cooked	9, 59.18, <0.0001	1, 2.67, 0.1027	5, 26.04, <0.0001	7, 6.80, <0.0001	25, 0.51, 0.9788
Fruity	9, 44.72, <0.0001	1, 0.73, 0.3932	5, 71.04, <0.0001	7, 0.30, 0.9533	25, 0.27, 0.9999
Strawberry	9, 19.86, <0.0001	1, 0.57, 0.4496	5, 185.84, <0.0001	7, 2.00, 0.0523	25, 1.64, 0.0262
Chocolate	9, 8.86, <0.0001	1, 0.56, 0.4548	5, 475.55, <0.0001	7, 3.87, 0.0004	25, 3.94, <0.0001
Vanilla	9, 24.17, <0.0001	1, 0.01, 0.9293	5, 114.95, <0.0001	7, 5.94, <0.0001	25, 0.70, 0.8638
Green	9, 31.61, <0.0001	1, 0.85, 0.3557	5, 4.59, 0.0004	7, 0.80, 0.5861	25, 1.14, 0.2926
Oxidized	9, 29.17, <0.0001	1, 0.68, 0.4093	5, 22.82, <0.0001	7, 0.67, 0.6961	25, 0.69, 0.9652
Sulfur	9, 32.32, <0.0001	1, 3.09, 0.0791	5, 6.84, <0.0001	7, 2.23, 0.0301	25, 1.14, 0.2914
Dirty	9, 14.88, <0.0001	1, 0.25, 0.6165	5, 11.67, <0.0001	7, 1.58, 0.1392	25, 2.59, <0.0001
Chemical	9, 21.55, <0.0001	1, 0.07, 0.7970	4, 40.57, <0.0001	7, 0.99, 0.4337	25, 1.73, 0.0150
Lactones	9, 25.29, <0.0001	1, 0.59, 0.4430	5, 13.54, <0.0001	7, 5.11, <0.0001	25, 0.79, 0.7543
Other off	9, 12.33, <0.0001	1, 0.29, 0.3926	5, 12.32, <0.0001	7, 9.03, <0.0001	25, 4.07, <0.0001

Change in each formulation over time

The changes in flavor attributes with storage time in our milk formulations are listed in Table 17. The overall flavor attribute ratings for each formulation are compared in Table 18. Unf-Uns did not differ significantly between weeks 1 and 6 in any attribute other than strawberry flavor. Although the milk was unflavored, panelists found that the strawberry flavor in the Unf-Uns milk decreased significantly between weeks 1 and 6 (Table 17). However, the average rating of strawberry flavor was 0.07 on a 15 point scale (Table 18), and as such is such a small number that further discussion is unnecessary.

Van milk decreased over time in many of the sensory attributes monitored, e.g. sweet, malty, butter/diacetyl, cream/fresh dairy, cooked/caramel, fruity, strawberry, vanilla, chemical/medicinal, and lactone/coconut attributes. Only the perception of the sour increased over weeks 1 to 8 (Table 17). Fruity, strawberry, chemical/medicinal, lactone/coconut and sour attribute changes, although significant, were not important as they were all below 1.0 on the 15 point scale (Table 18). Of these sensory notes, sweet, malty, butter, cooked, vanilla, and cream attributes can all be associated with the vanilla flavor itself. Strohman (Chapter 2) also found that the perception of vanilla flavor decreased with time in stored vanilla flavored milk.

Table 17: The effect of time on flavor attribute changes within each flavored and/or colored milk formulation

Attribute	Unf-Uns (1-6 wks)	Van (1-8 wks)	Choc (1-6 wks)	StCol (1-6 wks)	StFlv (1-6 wks)	StFlvPtt (1-6 wks)
Sweet	NS	p = 0.0064, decrease	p = 0.0077, decrease	NS	NS	NS
Sour	NS	p = 0.0003, increase	p < 0.0001, increase	p < 0.0001, increase	NS	p < 0.0001, increase
Salty	NS	NS	NS	NS	NS	NS
Bitter	NS	NS	NS	p = 0.0023, increase	NS	NS
Umami	NS	NS	NS	NS	NS	NS
Malty	NS	p = 0.0078, decrease	NS	NS	NS	NS
Butter/ diacetyl	NS	p = 0.0064, decrease	NS	NS	NS	NS
Cream/ fresh dairy	NS	p = 0.0002, decrease	p < 0.0001, decrease	p < 0.0001, decrease	p = 0.0127, decrease	p = 0.0013, decrease
Cooked/ caramel	NS	p = 0.0040, decrease	NS	NS	NS	NS
Fruity	NS	p = 0.0210, decrease	NS	NS	NS	NS
Strawberry	p = 0.0040, decrease	p = 0.0213, decrease	NS	p < 0.0001, decrease	NS	NS
Chocolate	NS	NS	p = 0.0002, decrease	NS	NS	NS
Vanilla	NS	p = 0.0123, decrease	p = 0.0059, decrease	NS	p = 0.0033, decrease	NS
Green	NS	NS	p = 0.0101, increase	NS	NS	NS
Oxidized/ cardboardy	NS	NS	p = 0.0010, increase	p = 0.0262, increase	NS	NS
Sulfur/ eggy	NS	NS	p = 0.0244, increase	NS	NS	NS
Dirty/smoky	NS	NS	p < 0.0001, increase	p = 0.0016, increase	NS	NS
Chemical/ medicinal	NS	p = 0.0460, decrease	p = 0.0211, increase	NS	NS	NS
Lactones/ coconut	NS	p = 0.0002, decrease	NS	NS	p = 0.0485, decrease	NS
Other off flavors	NS	NS	p < 0.0001, increase	p < 0.0001, increase	NS	NS

The ANOVA compared the mean ratings for weeks 1, 2, 3, 4, 5, and 6. A significant p value indicates that at least one of those mean ratings is different from the others. If significant changes in attribute, $p<0.05$ listed as well as if attribute increased or decreased between the first and last week. NS denotes comparisons in which changes were not significant at $p<0.05$. The ANOVA compared all of the weeks of each formulation to each other. The significant finding means that at least one of the intensity ratings for one of the weeks is different from the others. The increase or decrease noted is a comparison of only the first week and the last week intensity ratings.

Table 18: Influence of flavored milk formulations on sensory attribute ratings overall.

Attribute	Unf-Uns (1-6 wks)	Van (1-8 wks)	Choc (1-6 wks)	StCol (1-6 wks)	StFlv (1-6 wks)	StFlvPtt (1-6 wks)
Sweet	1.1[a]	6.6[d]	4.5[b]	6.0[c]	6.9[d]	6.6[d]
Sour	0.7[a]	0.7[a]	2.2[c]	1.5[b]	0.7[c]	0.8[c]
Salty	0.3[a]	1.2[c]	1.7[d]	1.0[b,c]	0.8[b]	0.7[b]
Bitter	0.7[b]	0.3[a]	3.4[c]	0.4[a,b]	0.5[a,b]	0.5[a,b]
Malty	0.3[a]	2.5[d]	2.0[c]	1.5[b]	1.1[b]	1.3[b]
Butter/ diacetyl	0.7[a]	1.7[c]	0.9[a,b]	1.5[c]	1.0[a,b]	1.1[b]
Cream/ fresh dairy	4.4[d]	3.2[c]	1.9[a]	2.8[b,c]	2.5[b]	2.5[b]
Cooked/ caramel	0.9[a]	3.0[c]	1.9[b]	1.8[b]	1.4[b]	1.6[b]
Fruity	0.1[a]	0.5[a]	0.2[a]	1.9[b]	3.7[c]	3.4[c]
Strawberry	0.1[a]	0.3[a]	0.1[a]	1.9[b]	6.2[c]	5.9[c]
Chocolate	0.1[a]	0.2[a]	6.7[b]	0.2[a]	0.1[a]	0.1[a]
Vanilla	0.2[a]	4.5[c]	0.5[a,b]	1.0[b]	0.4[a]	0.5[a,b]
Green	0.5[a,b]	0.4[a]	0.4[a]	0.5[a,b]	0.8[b,c]	0.9[c]
Oxidized/ cardboardy	2.2[c]	0.5[a]	1.2[b]	0.8[a,b]	0.4[a]	0.4[a]
Sulfur/ eggy	0.4[a]	0.6[a]	0.4[a]	1.0[b]	0.3[a]	0.3[a]
Dirty/smoky	1.1[b]	0.6[a]	1.5[c]	0.6[a]	0.4[a]	0.4[a]
Chemical/ medicinal	0.1[a]	0.4[a]	0.5[a,b]	0.8[b]	1.8[c]	1.9[c]
Lactones/ coconut	0.4[a]	0.8[b]	0.4[a]	0.9[b]	0.8[b]	0.9[b]
Other off flavors	0.8[b]	0.6[a,b]	1.3[c]	0.9[b]	0.2[a]	0.2[a]

Each milk formulation is compared with every other milk formulation. Means within each attribute with different letters are significantly different than each other at $\alpha=0.05$ using Student-Newman-Keuls Test. The numbers in the table are the means for each formulation including all of the weeks listed in parentheses. Each value is an average of 2 ratings each by ten panelists for each week of storage (n=120 for all milks except Van where n=160).

Choc showed decreases over time in four sensory notes (sweet, cream/fresh dairy, chocolate, and vanilla attributes) and increased in several others (sour, green, oxidized/cardboardy, sulfur/eggy, dirty/smoky, chemical/medicinal, and other off flavors) (Table 17). Vanilla, green, sulfur/eggy, and chemical/medicinal attribute changes, although significant, were not important as they were all below 1.0 on the 15 point scale (Table 18). As Douglas et al. (2000) and Pearson and Marth (1990) found that cocoa powder increases the growth of microorganisms in milk, this may also be the cause of the sour, dirty/smoky, oxidized/cardboardy, and other off flavors. Both the Douglas study

(2000) and the Strohman study (Chapter 2) indicated that cocoa powder itself may accelerate the spoilage of chocolate milk. The perceived decrease in desirable flavors such as chocolate, cream/fresh dairy, and sweet attributes may be due to the increase in undesirable flavors masking the desirable flavors. This is commonly known as mixture suppression, in which mixtures of different tastes inhibit the ability to sense the tastes evenly (McBurney and Bartoshuk, 1973).

StCol decreased over time in two sensory notes (cream/fresh dairy and strawberry flavor) and increased over time in many others (sour, bitter, oxidized/cardboardy, dirty/smoky, and other off flavors) between weeks 1 and 6 (Table 17). Bitter, oxidized/cardboardy, dirty/smoky, and other off flavors attribute changes, although significant, were not important as they were all below 1.0 on the 15 point scale (Table 18). It should be noted that panelists' perception of less strawberry flavor over time paralleled the loss of pink color. Since no strawberry flavor was added to this sample, the color likely influenced the panelists' rating of this attribute. Lavin and Lawless (1998) found that color intensity indeed influenced sweet flavor ratings in fruit beverages. The reported increase in sour taste may partly be due to the fact that no flavor was added to mask this off-flavor, and partly because panelists may have expected to find flavor in the strawberry colored milk, even though none was added.

StFlv significantly decreased in three sensory notes (cream/fresh dairy, vanilla, and lactone/coconut) between weeks 1 and 6 (Table 17). Vanilla and lactone/coconut attribute changes, although significant, were not important as they were all below 1.0 on the 15 point scale (Table 18). Unexpectedly, although fresh, creamy milk notes decreased with time, strawberry flavor was unaffected. This was surprising as the chocolate flavor in Choc, vanilla flavor in Van, strawberry flavor in StCol decreased in intensity with time.

StFlvPtt significantly decreased in cream/fresh dairy notes and increased in the sour note between weeks 1 and 6 (Table 17). Sour note changes, although significant, were not important as it was below 1.0 on the 15 point scale (Table 18). Again strawberry flavor was unaffected; however, this is not as surprising, because the flavor had little time to interact with the milk matrix.

Comparing formulations

The sweet note was lowest in Unf-Uns milk, probably because no sugar was added (Table 18). The sweet note was highest in StFlv, Van, and StFlvPtt and lower in StCol, possibly because the flavors enhanced the sweetness (Table 18). This relates to Lavin and Lawless (1998) who noted that adding vanillin to milk influenced panelists to think the flavored milk was sweeter. Choc also was comparatively less sweet due to the addition of the bitter, unsweetened cocoa powder, which overpowered the sugar. Although the cocoa powder would have ordinarily have been paired with extra sugar, we chose to limit the variables in these experiments. Choc was considered most salty, sour and bitter, which was due to the unsweetened cocoa powder. StCol was sour probably due to the expectation of strawberry notes when none was added to mask such notes. Malty notes were highest in Van and of next highest intensity in Choc, both of which contained a smoky, malty vanilla flavoring (Table 18). Cream/fresh dairy notes were highest in the Unf-Uns and the StCol and lowest in Choc (Table 18). The “freshness” of unflavored milk was expected, since this quality is overpowered by popular flavorings as noted in Strohman et al. (Chapter 2). Cooked/caramel notes were of highest intensity in Van and of lowest intensity in the Unf-Uns (Table 18). This was expected as Unf-Uns did not contain sucrose to be heated during pasteurization. Also, caramels often contain sugar, milk, and vanilla (ingredients in Van), so vanilla flavor may be associated with caramel flavor. Fruity and strawberry notes were highest in intensity in the StFlv and the StFlvPtt; however, fruit and strawberry notes were higher in the StCol than in Van, Choc, and Unf-Uns (Table 18). The color may have influenced this rating in the StCol. Strawberry notes were similar in the StFlv and StFlvPtt, which means that pasteurization did not decrease the strawberry notes as expected. As expected, chocolate notes were of highest intensity in Choc and vanilla notes were of highest intensity in Van (Table 18). Oxidized notes were highest in the Unf-Uns probably because of the lack of other flavor compounds to mask this off-flavor (Table 18). Dirty/smoky notes were highest in Choc and next highest in the Unf-Uns (Table 18). The bitterness of the cocoa powder and the lack of other strong flavors may have contributed to this. Chemical/medicinal notes were highest in the StFlv and StFlvPtt as the strawberry flavor itself was somewhat medicinal. As previously described, other off-flavors were highest in the Choc (Table 18).

Conclusions

Whereas we expected to find that all desirable characteristic flavors decreased with time, we found that chocolate and vanilla flavor did, indeed, decrease, but strawberry flavor did not. We expected that undesirable flavors would increase with time and found this to be correct. Further, the experiments seemed to show that strawberry color had a flavor component in and of itself, and it is well known that color affects flavor judgments. Although we thought pasteurization would decrease the intensity of strawberry flavor in milk, we found this to be untrue.

Future Research

To limit the scope of our research, our future work will limit the flavored milks under observation to just strawberry. We chose strawberry because chocolate milk (1) has been studied more than strawberry milk, (2) does require the addition of more sugar to be comparable, and (3) has more microbial concerns. For future research, we want to target a younger audience, since that is the main group of flavored milk drinkers. We want to gather data on liking in addition to descriptive analysis. We will also use instrumental analysis to identify compounds.

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Chapter IV:

The Effect of Storage Time, Color, and Flavor on Liking of Strawberry Flavored Milk

Introduction

Growing children are in need of vitamins, minerals, and high quality proteins to build strong bones and healthy bodies. Fluid milk is a good source of these nutrients (Committee on School Health, 2004). Although milk once was a staple in children's diets, overall beverage milk consumption has decreased 13% between the 1982 and 2007 (27 gallons per capita to 24 gallons per capita) (USDA/ERS). Instead of choosing nutrient dense beverages such as milk, children are drinking sodas (an increase from about 22 gallons per capita in 1970 to 39 gallons per capita in 1995) (Committee on School Health, 2004; Mathematica Policy Research, 2001; United States Department of Agriculture, 1997). While flavored milk consumption increased by 51% (1.2 gallons per capita to 1.7 gallons per capita) between 1986 and 2006, this was small in absolute numbers compared to soda consumption (United States Department of Agriculture, 1997; USDA/ERS). This suggests two opportunities for those concerned with child nutrition: improve the flavor and availability of flavored milk to compete with soda as a beverage for children or improve the nutrient value of soda. We will address the former in this paper.

Many food products are identified by their distinctive flavors. Milk, however, is characterized by a very bland flavor. This does not mean that milk lacks flavor. The lactose in milk makes it slightly sweet, while salts make it slightly salty (Walstra et al., 1999). The pasteurization of milk gives it a slight to moderate cooked flavor (depending on the temperature/time conditions) (Badings, 1991). Unflavored milk is usually described favorably as, simply, *milk* or unfavorably in much more detail. In contrast, flavored milk starts with a flavor noticeably different than just *milk*. Therefore, unfavorable flavors in flavored milk may be due to decreases in intensity of the added flavor or the increases in intensity of undesirable flavors. Research has been conducted on the flavor of unflavored milk (especially looking at flavors caused by light-oxidation, staling, and feed taints), but little has been done to look at flavored milk, especially milk flavors other than chocolate.

Strawberry flavors consist primarily of esters and furanones (Reineccius, 1994). Esters tend to be chemically inert in most food environments. However, they are readily hydrolyzed by enzyme attack (e.g. esterases). Esterases are indigenous in milk and related to microorganisms. Thus, the types of microorganisms and their populations in the milk, in addition to the thermal process (which inactivate the esterases) can influence the stability of these compounds. Furanones are relatively susceptible to oxidation/reduction reactions, so their stability is also in question. The perception of strawberry flavor may be related to more than the flavor itself. Color's influence on flavor is discussed in Chapter 3. In addition, Davidson (1999) found that perception of flavor in chewing gum was related to the amount of sweetener present. We may find a similar correlation in flavored milk. Establishing reliable measures for the subjective experience of flavor is essential to this research.

Although one may have descriptive sensory data on milks during storage (as discussed in Chapter 3), hedonic tests are needed to determine the actual acceptability of these well described milks. Liking can be determined by tasting milk samples and rating them on a hedonic scale. A common structured 9-point scale lists choices ranging from "dislike extremely" to "like extremely" (Lawless and Heymann, 1999). Scales for children sometimes have fewer choices or different words. For example, the P& K Verbal Scale for Use with Children has anchors ranging from "super bad" to "super good" (Kroll, 1990). Although descriptive panels are considered trained instruments, liking panels are more similar to opinion polls and, therefore, require more participants to be statistically viable.

In addition to sensory methods of monitoring flavor changes, instruments can be used to monitor changes in quantities of the flavor compounds themselves. This study used gas chromatography (GC) coupled with mass spectrometry (MS) to analyze samples collected with SPME techniques. A more thorough discussion of GC/MS/SPME can be found in Chapter 2.

We intended to chemically analyze strawberry milk over time for flavor compounds and to compare this with sensory data collected from a panel of college students. Since children are the target audience for flavored milk, we worked with a test panel comprised of children to see if adding strawberry flavor to milk would increase milk favorability.

We hypothesized that children would rate strawberry flavored milk stored for over 4 weeks as liked less than strawberry flavored milk stored for less than 4 weeks and that they would rate all strawberry flavored milk as liked more than strawberry colored milk.

Materials and Methods

Materials and Sources

Ingredients

Ingredients used are the same as in Chapter 3 except no cocoa powder was used.

Equipment

Equipment used was the same as in Chapter 3 with the addition of Gerstel Multipurpose Sampler MPS2 (Gerstel, Mülheim an der Ruhr, Germany); HP 6890 Series GC (Hewlett Packard, Palo Alto, CA); HP 6890 Mass Selective Detector (59-72A) (Hewlett Packard, Palo Alto, CA)

Materials

Materials used were the same as in Chapter 3 with the addition of J&W Scientific DB Wax 30m x 0.250mm x 0.25 µm column (Agilent Technologies, Inc., Santa Clara, CA); Supelco 75µm Carboxen™- PDMS SPME fiber assembly (Sigma-Aldrich, St. Louis, MO); SPME liner (78.5mm x 6.3mm OD, 1.5mm ID) (Sigma-Aldrich, St. Louis, MO); 2 oz. clear plastic cups (Commercial source)

Software

Software used was the same as in Chapter 3 with the addition of Wiley275 mass spectra library; R software (R Project, http://www.r-project.org)

Milk Ingredients

The flavored milk formulations used in this study are presented in Table 19.

Table 19: Modifications made to low-fat milk to create the sample formulations.

Sample	Flav1*	Col2*	FlavCol3*	Flav4	Flav5	Flav6*	Flav7	Flav8
Strawberry Flavor and Color [a]	100%	0%	~15%	100%	100%	100%	100%	100%
Strawberry Color without Flavor [b]	0%	100%	~85%	0%	0%	0%	0%	0%
Weeks in storage	1	2	3	4	5	6	7	8
2% fat milk	x	x	x	x	x	x	x	x
Gum blend [c]	x	x	x	x	x	x	x	x
Sucrose [d]	x	x	x	x	x	x	x	x
Vitamins A & D3	x	x	x	x	x	x	x	x

* denotes milk that were tasted
[a] Natural Strawberry Flavor WONF with artificial color NV-32,294 (0.97% by weight)
[b] artificial color NV-32,294 (0.97% by weight)
[c] TIC Pretested® Dairy Blend Chocolate 46 (0.80% by weight)
[d] sucrose (5% by weight)

Sample preparation/Processing

Raw, whole fat, cow's milk was obtained from Dairy Farmers of America and separated into cream and skim milk in the University of Minnesota pilot plant using a Westphalia MP1254 separator. Both cream and skim milk were analyzed by IR for fat content by DQCI Services (Mounds View, MN). Raw skim milk and cream were mixed to reach a final fat level of 2% by weight. Eight gallons (68.8 lbs.) of milk were used of each week of milk processing. The raw milk, color/flavor combinations (see Table 19), sucrose, TIC Pretested® Dairy Blend Choc-46, a commercial mixture of vitamins A and D3 were mixed using a Lightenin Mixer, Rotation, Model C4. As listed in Chapter 2, TIC Pretested ® Dairy Blend Choc-46 is a mixture of gums used for thickening chocolate milk. The formulation does not contain any chocolate flavor. The milks were pasteurized (89°C for 13 sec using a Microthermics® UHT/HTST Lab Electric Model 25HV Hybrid) and homogenized (1500psi) using a GEA Niro Soavi homogenizer, Type NS2006H, CP-1, S06H-4796. Then we filled (using a Microthermics® Clean Fill Hood & Sterile Product Outlet) 16oz. opaque HDPE containers (cleaned with 200ppm hypochlorite solution and rinsed with distilled, autoclaved water) with the formulations. The filled bottles were capped and placed on ice to continue cooling the milk (that was still warm from pasteurization). The bottles were then transferred to a refrigerator (4-7°C) to be stored until analysis. Milk samples were prepared weekly for eight weeks and stored so they could all be analyzed at the same time.

Gas Chromatography/Solid Phase Microextraction (GC/SPME)

For each stored week of milk, three 4 ml aliquots were taken from two bottles of milk (1 from one bottle and 2 from another bottle) and poured into separate 20ml vials. Sample order was randomized within each replication using a random number table. Vials were warmed to 45°C and held for 45 min using a Gerstel Multipurpose Sampler MPS2 prior to 10 min of sampling of the headspace with a Supelco 75µm Carboxen™-PDMS SPME fiber assembly. The SPME fiber was then desorbed into the GC inlet (225ºC), which had a SPME liner (78.5mm x 6.3mm OD, 1.5mm ID), at a flow rate of 25.4ml/min (splitless for the first minute and split after that). A J&W Scientific DB Wax 30m x 0.250mm x 0.25 µm column was used in a HP 6890 Series GC with an HP 6890 Mass Selective Detector (59-72A) and the GC oven heated from 40°C to 200°C at a rate of 5°C/min. Compounds were tentatively identified by comparing the mass spectra found in analysis with a Wiley275 mass spectra library. Statistics were calculated using R software.

Microbial Testing

Microbial testing procedures were the same as in Chapter 3.

Descriptive Analysis

First, a panel of ten students created a list of twenty attributes as described in Chapter 2. Then a second group of panelists was trained on these attribute as described in Chapter 3.

Children's Liking Tests

Children (aged 7 to 13) were recruited by Food Perspectives, Inc. (Plymouth, MN) for this study. One hundred eleven children were presented milk samples Flav1, Col2, FlavCol3, and Flav6, one sample at a time (4 ounces of each sample) in clear plastic cups. They were then asked to rate the milks on 7 scales: liking of appearance (1 to 7), liking of color (1 to 7), liking of taste (1 to 7), strength of strawberry flavor (1 to 3), strength of sweetness (1 to 3), overall like or dislike (1 to 5), and likelihood of purchase (1 to 2). For each question, 1 indicates the least liked or the lowest strength. The questions were read aloud to each group of children and each child checked the box next to the appropriate answer. Paper ballots were used. Statistical analysis was calculated using SAS software.

Statistical Design

This study was designed to be a factorial of 1x4 with tasting done of milk stored for 1, 4, 5, and 6 weeks. Unfortunately, due to miscommunication over microbial counts, only weeks 1, 2, 3, and 6 were tasted. Also, all of the milk formulations were intended to get the same amount of milk flavor added. The bottle was misread in weeks 2 and 3 and color was added instead of some flavor. As it is, there is no factorial. The purpose of this study was to determine at what storage time liking decreases sharply. However, the analysis will examine how color and flavor as well as storage time influence liking of strawberry flavored milk by children. The GC/MS statistical model was based on changes in compound peak areas depending on time stored. The trained panel statistical model includes the influence Judge (panelist – 9 degrees of freedom), Rep (repetition of panelist testing of each treatment – 1 degree of freedom), and Milk (milk treatment – 3 degrees of freedom) on sensory attribute rating changes. The statistical model for the children looked for differences in answers to seven questions. Four milk treatments (degrees of freedom = 3) were tasted and rated by 111 children (error degrees of freedom = 330).

Results and Discussion

We set out to chemically analyze the strawberry flavor components of strawberry milk over time and compare this to sensory panel data. Because children are the target market of flavored milk sales, we also had a panel of children rating the liking of these milks. We chose to examine strawberry flavored milk rather than chocolate or vanilla flavored milk. Chocolate milk requires the addition of more sugar to be comparable, the microbial counts seem to go up much faster, and vanilla flavored milk compounds could not be tested using SPME because the primary flavorant, vanillin, is not sufficiently volatile, thus we determined strawberry flavored milk was best for this analysis.

GC/SPME

The aroma compounds one extracts in flavor analysis depends largely on the techniques used and this is especially true with Solid Phase Microextraction (SPME). A Carboxen/PDMS fiber was best for this study since it is known to be most effective in extracting many low molecular weight volatile compounds (Marsili, 1999). A list of compounds in the milk samples tentatively identified by mass spectrometry as well as some odor descriptions associated with those compounds and the significance of differences found in ANOVA analysis can be found in Table 20. Compounds showing significant differences will be discussed first. Only four flavor compounds changed significantly over time in milk with flavor added. These changes are displayed in figures 1 (acetone), 2 (ethyl lactate), 3 (acetic acid), and 4 (butyric acid).

Acetone, a compound commonly found in fresh milk, decreased with storage time (Figure 1). Wadsworth and Bassette (1985) also found acetone to decrease with storage time. Ethyl butyrate and *cis*-3-hexenol were present in greater amounts in the milks where the full amount flavor was added because these components are part of the flavor, but did not change otherwise during storage (Table 20). This is in contrast to Siegmund et al. (2001), who studied a stored strawberry drink (containing no milk) and found that ethyl butyrate and *cis*-3-hexenol concentrations decreased with storage. However, they did not study strawberry flavor in milk and their samples were stored at 37°C as opposed to refrigeration temperatures. Ethyl lactate is another strawberry flavor component, and it remained the same throughout the sampling time except for a high point in week 5 (Figure 2). The week 5 milk seems to have been materially different in this respect.

Table 20: Significant changes in tentatively identified of compounds in strawberry flavored and/or colored milk by mass spectrometry over time

Compound/Milk	Odor Description[a]	F, df1, df2 all samples	p value[b] all samples	F, df1, df2 only Flav samples	p value[b] only Flav samples
acetaldehyde	pungent, ether, fruity	0.91, 5, 7	NS	1.22, 4, 6	NS
acetone	apple, pear, grape, pineapple, ether	3.70, 7, 16	**<0.05**	6.08, 5, 12	**<0.01**
ethyl acetate	dry, fruity, musty, pineapple	1.80, 5, 9	NS	1.99, 4, 8	NS
2-butanone	acetone-like, ethereal, fruity, camphor	2.24, 7, 15	NS	2.22, 5, 12	NS
2-methyl butanal	musty, cocoa, coffee, nutty	1.86, 4, 8	NS	1.86, 4, 8	NS
ethanol[c]	strong, ether, medicinal	0.84, 7, 14	NS	1.67, 5, 11	NS
diacetyl	strong butter, sweet, creamy, pungent, caramel	0.87, 5, 11	NS	1.05, 4, 10	NS
ethyl butyrate[c]	fruity, juicy, fruit, pineapple, cognac	23.32, 6, 14	**<0.001**	1.89, 5, 12	NS
2-heptanone[c]	fruity, spicy, sweet, herbal, coconut, woody	2.85, 6, 13	NS	0.35, 5, 12	NS
butyl butyrate[c]	fine, fruity, pineapple, sweet	0.43, 4, 10	NS	0.43, 4, 10	NS
ethyl hexanoate[c]	sweet, fruity, pineapple, waxy, fatty, green banana	2.18, 4, 10	NS	2.18, 4, 10	NS
1-hydroxy-2-propanone		1.26, 6, 13	NS	0.89, 5, 11	NS
cis-3-hexenyl acetate[c]	fresh green, sweet, fruity, apple, pear, melon, green banana	2.29, 4, 9	NS	2.28, 4, 9	NS
ethyl lactate[c]	sharp, tart, fruity, buttery, butterscotch	4.08, 5, 11	**<0.05**	4.08, 5, 11	**<0.05**
***cis*-3-hexenol[c]**	foliage green, fresh, oily, cut grass	4.13, 6, 13	**<0.05**	2.23, 5, 12	NS
acetic acid	sharp, pungent, sour, vinegar	3.43, 5, 11	**<0.05**	4.69, 4, 10	**<0.05**
butyric acid	sharp, dairy, cheese, butter, fruit	3.93, 6, 12	**<0.05**	283.59, 5, 10	**<0.001**
hexanoic acid	mild sour, fatty, sweat, cheese	0.73, 6, 10	NS	0.72, 5, 8	NS

[a] Odor descriptions were obtained from http://www.thegoodscentscompany.com.
[b] NS denotes that the p value was greater than 0.05 (α).
[c] denotes compounds in the strawberry flavor
* Each of 8 weeks of samples were analyzed in triplicate.

Acetic acid increased with storage time (Figure 3). Although acetic acid is sometimes added as part of a strawberry flavor, its increase with storage time suggests a microbial source. Butyric acid increased with time although peaks for weeks 2 and 8 were many times greater than the peaks for the other weeks (Figure 4). In the week 2 milk, color was added without flavor, so this could have resulted in more butyric acid being picked up by the SPME fiber, since the flavor compounds were not there to interfere. In the week 8 milk, this could be due to an increase in microorganism growth with time leading to lipolysis (Shipe et al., 1978).

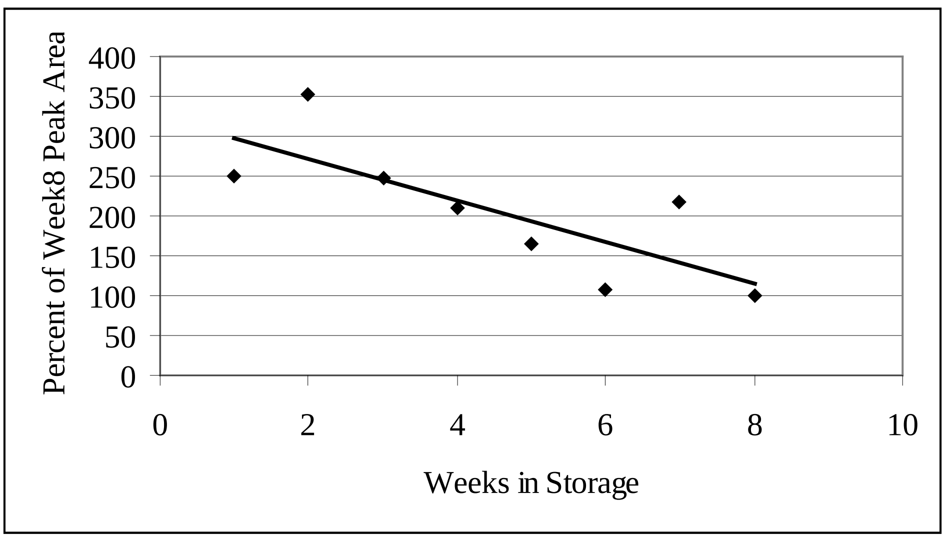

Figure 1: Influence of storage time on acetone concentrations in strawberry flavored milk.

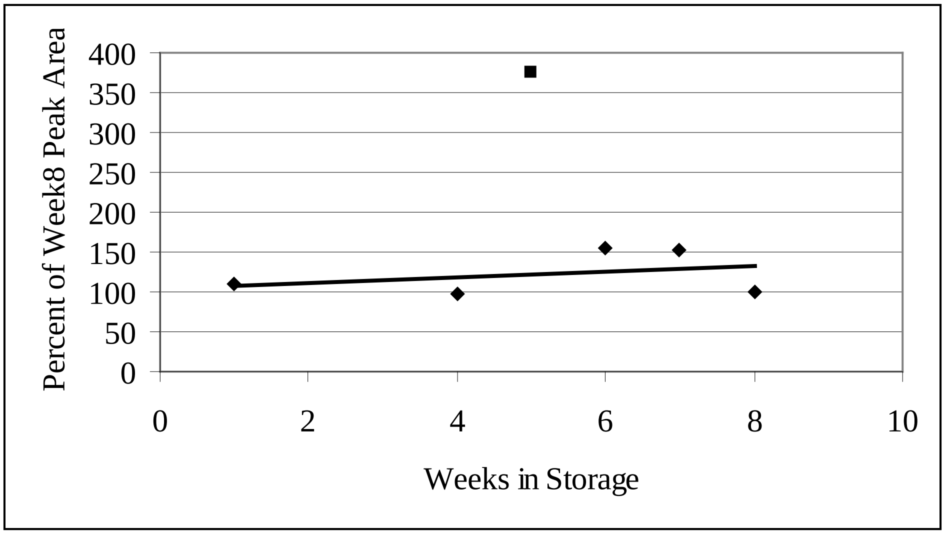

Figure 2: Influence of storage time on ethyl lactate concentrations in strawberry flavored milk.

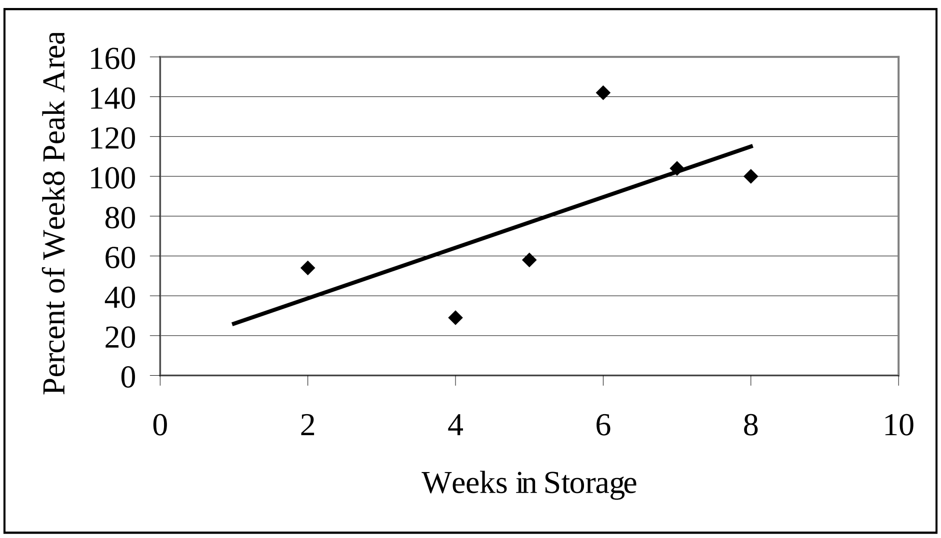

Figure 3: Influence of storage time on acetic acid concentrations in strawberry flavored milk.

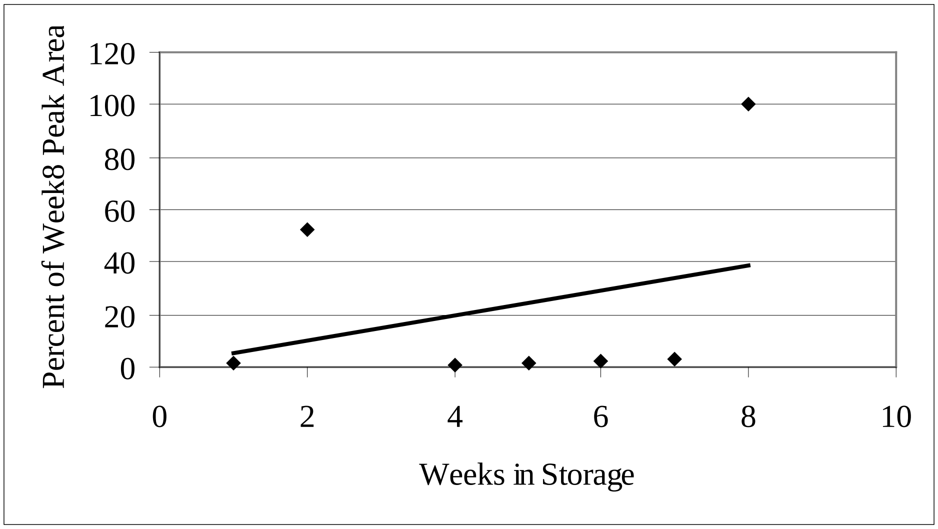

Figure 4: Influence of storage time on butyric acid concentrations in strawberry flavored milk.

We did not find any changes in the quantities of acetaldehyde, ethyl acetate, 2-butanone, 2-methyl butanal, ethanol, diacetyl, and hexanoic acid during storage. These compounds are regularly found in raw milk as they originate from cow feed (Toso et al., 2002; Kirk et al., 1968; Scanlan et al., 1968; Coulibaly and Jeon, 1992). In support, Wadsworth and Bassette (1985) did not find any changes in acetaldehyde with storage in their sterilized milk, however, Jaddou et al. (1978) found a decrease, but only in milk that was pasteurized for a longer time (90 sec vs. 3 sec). Jaddou et al. (1978) also found ethanol to increase initially and then decrease with storage. We may have found different results due to the fact that we processed our milk at a lower temperature for a shorter time and stored our milk at refrigeration temperatures rather than at ambient temperatures. 2-heptanone is a compound found in heated milk, but it was added to our milk as part of the strawberry flavor. Butyl butyrate, ethyl hexanoate, and *cis*-3-hexenyl acetate were also added as part of the strawberry flavor, but did not change in concentration during storage time. 1-hydroxy-2-propanone did not change with storage in this study.

Descriptive Analysis

By definition, the descriptive analysis panel was trained to act as an instrument rather than to give hedonic responses to milk flavors. Our descriptive analysis panel tasted all four milks that were presented to the children (discussed later). The statistical significance of differences in sensory attribute ratings are found in Table 21, but are not discussed. The intensity ratings on twenty sensory attributes of strawberry flavored and/or colored milk samples by the descriptive analysis panel are found in Table 22.

Table 21: Statistical significance of trained panel sensory testing on sensory attributes rating changes

Sensory Attribute	Judge (df, F, p)	Rep (df, F, p)	Milk (df, F, p)
Sweet	9, 42.54, <0.0001	1, 0.09, 0.7650	3, 9.73, <0.0001
Sour	9, 2.67, 0.0106	1, 0.07, 0.7985	3, 44.55, <0.0001
Salty	9, 5.70, <0.0001	1, 0.70, 0.4072	3, 0.19, 0.9042
Bitter	9, 4.55, 0.0001	1, 0.15, 0.6986	3, 5.30, 0.0025
Umami	9, 7.14, <0.0001	1, 0.04, 0.8433	3, 0.53, 0.6602
Malty	9, 10.42, <0.0001	1, 0.01, 0.9045	3, 2.13, 0.1049
Butter	9, 4.72, <0.0001	1, 0.41, 0.5238	3, 2.03, 0.1177
Cream	9, 2.77, 0.0082	1, 0.16, 0.6932	3, 5.24, 0.0026
Cooked	9, 16.52, <0.0001	1, 0.88, 0.3521	3, 7.16, 0.0003
Fruity	9, 14.18, <0.0001	1, 0.03, 0.8716	3, 5.42, 0.0021
Strawberry	9, 7.26, <0.0001	1, 0.14, 0.7089	3, 10.52, <0.0001
Chocolate	9, 19.18, <0.0001	1, 0.46, 0.5005	3, 0.98, 0.4060
Vanilla	9, 4.48, 0.0001	1, 0.03, 0.8726	3, 3.57, 0.0185
Green	9, 11.43, <0.0001	1, 0.06, 0.8026	3, 4.06, 0.0104
Oxidized/cardboardy	9, 26.42, <0.0001	1, 1.41, 0.2389	3, 2.17, 0.1000
Sulfur/eggy	9, 1.79, 0.0866	1, 1.37, 0.2465	3, 1.28, 0.2885
Dirty/etc.	9, 5.54, <0.0001	1, 1.29, 0.2601	3, 4.75, 0.0046
Chemical/medicinal	9, 9.83, <0.0001	1, 0.26, 0.6090	3, 8.82, <0.0001
Lactones/coconut	9, 6.73, <0.0001	1, 0.54, 0.4659	3, 5.35, 0.0023
Other off flavors	9, 3.17, 0.0031	1, 0.61, 0.4366	3, 7.47, 0.0002

Table 22: Influence of storage time and flavor addition on sensory attributes of milk

Attribute/Milk	Flav1[d,e]	Col2	FlavCol3	Flav6
sweet	6.8[c]	5.2[a,b]	5.8[b]	4.3[a]
sour	0.9[a]	0.6[a]	1.1[a]	5.4[b]
bitter	0.6[a]	0.4[a]	0.4[a]	1.4[b]
cream/fresh dairy	2.3[a,b]	3.4[b]	2.5[a,b]	1.3[a]
cooked/caramel	1.6[b]	1.8[b]	2.0[b]	0.7[a]
fruity	2.6[b,c]	1.1[a]	1.6[a,b]	2.9[c]
strawberry	4.8[b]	1.3[a]	1.3[a]	2.3[a]
vanilla	0.2[a]	0.7[a,b]	1.1[b]	0.1[a]
green	0.9[a]	0.5[a]	0.6[a]	1.7[b]
dirty/smoky/etc.	0.3[a]	0.4[a]	0.5[a]	1.2[b]
chemical/medicinal	1.7[a]	0.8[a]	0.8[a]	2.7[b]
lactones/coconut	1.2[a]	0.8[a]	0.5[a]	2.0[b]
other off flavor	0.5[a]	0.3[a]	0.4[a]	2.2[b]

[a-c] Different letters denote differences in mean ratings within rows (attributes) using the Student-Newman-Keuls Test within the method of least squares (α=0.05, error degrees of freedom=66). It has been assumed that this is a general linear model.
[d] Mean ratings by a 10 person trained panel with each treatment tested in duplicate. All ratings are on an unlabeled line scale (15 points in length).
[e] Flav1 milk contained the full amount of flavor and was stored for 1 week. Col2 milk contained only red coloring (no flavor) and was stored for 2 weeks. FlavCol3 milk contained approximately 1/3 of the proposed amount of flavor (the rest was added color) and was stored for 3 weeks. Flav6 milk contained the full amount of flavor and was stored for 6 weeks.

The panelists found the sweetness of the oldest milk to be significantly less than that of the freshest milk (Table 22). The strawberry intensity ratings in the week 1 milk were higher than in the week 6 milk even though the GC/MS results did not show differences in strawberry flavor components. This was likely due to the loss of the sweetness, reducing the perceived strawberry flavor. This is similar to Davidson et al (1999), who found that perception of flavor in chewing gum was related to the amount of sweetener present. The panelists also noted that green and chemical/medicinal notes, which are attributes also associated with strawberry flavor (Strohman et al., Chapter 2), increased with storage in the fully flavored milks. They found that fruity notes did not change in the fully flavored milks. A possible reason for this is the compounds panelists used to rate strawberry and fruity notes changed in quantities too minute to be picked up by the instrument, because human nose is often much more sensitive than instrumentation by about 10^4. Another possible reason for this is that decreases in sweet taste and cooked/caramel flavors were directly related to increases in many off-flavors (sour, bitter, dirty/smoky, and other off flavor notes). Known as mixture suppression, the sour and bitter notes may have interfered with the perception of the strawberry notes (McBurney and Bartoshuk, 1973). Oxidized notes did not change with time although it is a common off-flavor found in stored, unflavored milk (Shipe, 1980; Hansen, 1987). Our findings could be due to the strawberry flavor masking oxidized notes or to oxidation rates being reduced by our storage of milk at refrigeration temperature as opposed to many studies of shelf stable UHT milk (Jeon, 1976; Jeon et al., 1978).

Children's Liking Study

Children can be better encouraged to drink nutrient dense beverages if they enjoy the flavor. An objective of this study was to determine how variations influence how much children like the flavored milk. Unfortunately, milk from weeks 4, 5, 7, and 8 had high microbial counts and thus could not be tasted, so weeks 1, 2, 3, and 6 were used. A summary of 111 children's responses to study questions is presented in Table 24. The statistical significance of these differences is listed in Table 23.

Table 23: Statistical differences in liking of strawberry flavored milk by 111 children

Question Asked and Rating Scale	df1, df2	F value	p value
"What do you think about how this strawberry milk looks?" with 1 being "really bad" and 7 being "really good."	**3, 330**	**20.16**	**<0.0001**
"What do you think about the color of this strawberry milk?" with 1 being "really bad" and 7 being "really good."	**3, 330**	**32.43**	**<0.0001**
"How is the taste of this strawberry milk?" with 1 being "really bad" and 7 being "really good."	**3, 330**	**15.40**	**<0.0001**
"How is the sweetness of this strawberry milk?" with 1 being "not sweet at all" and 3 being "very sweet."	**3, 330**	**2.40**	**0.0682**
"Thinking about everything, how much do you like or dislike this strawberry milk?" with 1 being "dislike a lot" and 5 being "like a lot."	**3, 330**	**13.39**	**<0.0001**
"Would you ask your parent(s) to buy this strawberry milk for you?" with 1 being "no" and 2 being "yes."	**3, 330**	**6.46**	**0.0003**

Table 24: Influence of storage time and flavor addition on liking of strawberry flavored milk by children

Question Asked and Rating Scale	Flav1 [d]	Col2	FlavCol3	Flav6
"What do you think about how this strawberry milk looks?" with 1 being "really bad" and 7 being "really good."	5.7 [c] *	5.6 [b,c]	5.4 [b]	4.6 [a]
"What do you think about the color of this strawberry milk?" with 1 being "really bad" and 7 being "really good."	5.6 [b]	5.5 [b]	5.5 [b]	4.2 [a]
"How is the taste of this strawberry milk?" with 1 being "really bad" and 7 being "really good."	4.6 [b]	4.2 [b]	4.2 [b]	3.2 [a]
"How much strawberry flavor is in this strawberry milk?" with 1 being "very little strawberry flavor" and 3 being "lots of strawberry flavor."	2.2 [b]	1.8 [a]	1.9 [a]	1.9 [a]
"How is the sweetness of this strawberry milk?" with 1 being "not sweet at all" and 3 being "very sweet."	2.2 [b]	2.1 [a,b]	2.1 [a,b]	2.0 [a]
"Thinking about everything, how much do you like or dislike this strawberry milk?" with 1 being "dislike a lot" and 5 being "like a lot."	3.3 [c]	3.0 [b]	3.1 [b,c]	2.4 [a]
"Would you ask your parent(s) to buy this strawberry milk for you?" with 1 being "no" and 2 being "yes."	1.4 [b]	1.4 [b]	1.4 [b]	1.2 [a]

[a-c] Different letters denote differences in mean ratings using the Student-Newman-Keuls Test within the method of least squares (α=0.05, error degrees of freedom=66). It has been assumed that this is a general linear model.
[d] Flav1 milk contained the full amount of flavor and was stored for 1 week. Col2 milk contained only red coloring (no flavor) and was stored for 2 weeks. FlavCol3 milk contained approximately 1/3 of the proposed amount of flavor (the rest was added color) and was stored for 3 weeks. Flav6 milk contained the full amount of flavor and was stored for 6 weeks.

When asked about how the milk looks and how much they liked the color of the milk, kids liked the looks of Flav1, Col2, and FlavCol3 better than milk Flav6 (Table 24). This is not a surprising result as Flav1, Col2, and FlavCol3 were pink in color, while Flav6 was white. FD&C Red #40 is relatively stable at varied pHs, but is susceptible to oxidation. It is likely that microbial activity caused oxidation of the color rendering it colorless. This finding of loss of color leading to loss of liking can be compared to a study by Lavin and Lawless (1998), who found that color intensity of fruit beverages influenced perception of sweetness.

When asked about the taste of the strawberry milks, children liked Flav1, Col2, and FlavCol3 better than Flav6 (Table 24). It should be noted that the descriptive panel rated Flav6 as more sour and bitter than the other milks. This is probably the main reason for the children's choice. Another possibility is that the panel likely used color to judge liking. This is in contrast with the work of Tuirila-Ollikainen et al. (1984), who found that liking was based on sweetness and presence of flavor rather than on color in raspberry and pear flavored drinks. However, our finding is similar to that of Pangborn and Hansen (1963), who found that participants could better identify differences in sweetness or sourness in pear nectar when color was absent as well as to that of Calvo et al. (2001), who found that flavor ratings increased as color intensity increased in fruit flavored yogurts even when sweetener and flavor contents remained unchanged.

When asked about the strawberry flavor in the strawberry milks, the children thought that Flav1 contained significantly more strawberry flavor than did the other milks (Table 24). Even though the liking of the flavor did not depend much on the strawberry flavor, the children did notice the absence or deterioration of the strawberry flavor.

When asked about the sweetness of the strawberry milks, the young study participants liked Flav1 better than Flav6 (Table 24). Flav1 and Flav6 were made with the same formulation, but stored for different lengths of time before tasting. One likely cause for this is an increase in off-flavors that might overpower the sweet taste. However, as with the previous question, color may be a factor. This is similar to the findings of Roth et al. (1988), who found that lemon beverage was rated as sweeter when more yellow coloring was added (but only up to a certain level of sweetness) and of Pangborn (1960), who found red and orange colorings influenced sweetness ratings in fruit nectars. However, somewhat contrary to our findings, Pangborn also found that colors did not influence sweetness ratings when the nectars were unflavored.

When asked how much they liked or disliked the strawberry milks, children liked Flav1 better than milk Col2 and also liked all other milks (Flav1, Col2, and FlavCol3) better than Flav6 (Table 24). Here flavor made a little difference, but extended storage made a difference. This supports the hypothesis that extended storage (more than 4 weeks) will decrease liking. FlavCol3 contained a slight bit of flavor, while Col2 did not (Table 19). FlavCol3 was not liked significantly less than Flav1, but Col2 was. FlavCol3 and Flav1 both contained some flavor, while Col2 did not, so here flavor was a contributor to liking. The ratings for most of these milk questions fall in the middle of the scale (neither like nor dislike).

When asked if they would ask their parents to buy this strawberry milk for them, the children gave more favorable ratings to Flav1, Col2, and FlavCol3 than Flav6 (Table 24). No (1) and yes (2) were their only two options. Flav6 was liked less for flavor, color, and sometimes for sweetness, but a little more than 20% of the kids would still ask their parents to purchase Flav6. Also, even though fewer kids would request Flav6 than would ask their parents to buy the other milks, only about 40% of the kids would request any of the milks.

Conclusions

According to our instrumental results, there were no changes in the strawberry flavor components that we were able to measure during storage. There were changes in other compounds though, as acetone levels decreased and acetic acid and butyric acid levels increased. By contrast, our sensory descriptive analysis panel results show that the strawberry flavor character decreased with time. This finding means that either the panelists were better able to rate strawberry flavor because they picked up aromas that the instrument was unable to detect or that the diminishing strawberry flavor ratings were based on the development of off-flavors rather than the disappearance of the major components of the strawberry flavor. Other studies have concluded that the latter is the most likely reason. Children's liking of flavored milk was in inverse relationship to the length of time in refrigerated storage, however, color and sweetness levels compensated for lack of strawberry flavor in some of the beverages. In summary, instrumental results indicated that strawberry flavor did not change with time, while the descriptive analysis panel indicated that it did. The children noticed, in the milks stored for less than 4 weeks, that the milk with the most strawberry flavor had more strawberry flavor than the one without any strawberry flavor, but did not like the taste of the other two milks significantly less than the one with the most strawberry flavor. The children's liking panel implied that pleasant strawberry flavor intensity was not the foremost deciding factor in their liking of strawberry milk. Storage time had the greatest influence on liking.

Future Research

Next, we chose to test various methods of processing and addition of ingredients to delay the appearance of off-flavors. It has already been determined that color influences flavor, so we did no additional studies on that, but focused on quantities and quality of flavor instead.

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Chapter V:
The Effect of Ingredients, Processing Conditions, and Time of Flavor Addition on Flavors in Strawberry Flavored Milk

Introduction

For nearly one hundred years, researchers have attempted to use chemical and sensory analysis to define the flavors of milks, creating a multiplicity of terms to describe attributes that are most often undesirable as opposed to being desirable. The implicit hope was to be able to determine the causes of undesirable flavors so as to eliminate them. More recently, sophisticated laboratory instrumentation such as gas chromatography and mass spectrometry have been developed and used for this purpose.

While the purposeful addition of flavors such as chocolate and strawberry radically changes the flavor of milk, the base product remains the same. For that reason, all the identified hazards to the flavor quality of regular milk remain with flavored milks – the cow's diet, health, and environment, transportation, temperature, time, heat and method of pasteurization – all can have a deleterious effect (Shipe, 1980). Research has been conducted on the flavor of unflavored milk (especially looking at flavors caused by light-oxidation, staling, and feed taints), but little has been done to look at flavored milk, especially milk flavors other than chocolate.

Descriptive analysis and gas chromatography were discussed in Chapter 2. Strawberry flavor components were discussed in Chapter 4.

We chose to vary some ingredients and processing parameters and monitor these changes in quality and persistence of flavor. We correlated flavor attributes from a trained sensory panel with measurable aroma components. We hypothesized that (1) adding flavor post pasteurization is more of a factor in determining flavor quality than storage of flavor in milk, (2) adding extra flavor or HFCS will prolong the shelf life as it will decrease flavor deterioration, and (3) adding vanilla flavor or removing air from the milk will extend shelf-life by decreasing microbial growth.

Materials and Methods

Materials and Sources

Ingredients

Ingredients are the same as in Chapter 3 except that only Vanilla flavor was used. Instead the following ingredients were used to make a strawberry flavor: Maltol (3-hydroxy-2-methyl-4H-pyran-4-one) (Sigma-Aldrich, Milwaukee, WI); aldehyde C16 (ethyl 3-methyl 3-phenyl glycidate) (Sigma-Aldrich, Milwaukee, WI); *cis*-3-hexen-1-ol (Sigma-Aldrich, Milwaukee, WI); Ethyl butyrate (Sigma-Aldrich, Milwaukee, WI); Isoamyl isovalerate (3-methylbutyl-3-methyl butyrate) (Sigma-Aldrich, Milwaukee, WI); Ethyl isovalerate (ethyl-3-methyl butyrate) (Sigma-Aldrich, Milwaukee, WI); Ethyl hexanoate (Sigma-Aldrich, Milwaukee, WI); γ-decalactone (Sigma-Aldrich, Milwaukee, WI); 2-methyl butyric acid (Sigma-Aldrich, Milwaukee, WI); Propylene glycol (Sigma-Aldrich, Milwaukee, WI). Red food coloring (McCormick & Co., Inc., Hunt Valley, MD) was also added.

Equipment

Equipment used was the same as in Chapter 4 with the addition of Sunset Milk Cooler (Sunset Electric Co., Seattle); Rogers Pan Single Effect Evaporator (C. E. Rogers Company, Mora, MN

Materials

Materials used are the same as in Chapter 4.

Software

Software used was the same as in Chapter 4 with the exception that the program R software was not used.

Milk Ingredients

The sample formulations used are described in Table 25. The composition of the strawberry flavor indicated in formulations in Table 25 is described in Table 26.

Table 25: Modifications made to low-fat milk to create the sample formulations.[a]

	Sweetener	Time of adding flavor	Amount of strawberry flavor added[b]	Pasteurization temperature	Other ingredients	Other treatments
HFCS	344g 55DE HFCS[c] /gal milk	Prior to pasteurization	15.56ml/gal	89°C, 13s	N/A	N/A
Vacuum	189g sucrose/ gal milk	Prior to pasteurization	15.56ml/gal	89°C, 13s	N/A	30 min in vacuum pan[d]
High Temp	189g sucrose/ gal milk	Prior to pasteurization	15.56ml/gal	99°C, 13s	N/A	N/A
Add Van	189g sucrose/ gal milk	Prior to pasteurization	15.56ml/gal	89°C, 13s	6g vanilla flavor/gal[e]	N/A
Control	189g sucrose/ gal milk	Prior to pasteurization	15.56ml/gal	89°C, 13s	N/A	N/A
2x Flav	189g sucrose/ gal milk	Prior to pasteurization	31.12ml/gal	89°C, 13s	N/A	N/A
Flav Lat	189g sucrose/ gal milk	After pasteur-ization, just prior to testing	15.56ml/gal	89°C, 13s	N/A	N/A
Flav Mid	189g sucrose/ gal milk	Shortly after pasteurization	15.56ml/gal	89°C, 13s	N/A	N/A

[a] All milks contained 2% milk fat, TIC Pretested® Dairy Blend Choc-46 (TIC Gums, Belcamp, MD; salt, corn starch, carrageenan [standardized with potassium chloride and dextrose], maltodextrin, and tetrasodium pyrophosphate [0.80% by weight], 0.5ml McCormick Red Food Coloring per gal milk, and 0.04ml vitamins/gal milk. All proportions are weight or volume of ingredients per weight or volume of milk (without the added ingredients).
[b] Strawberry flavor was prepared following the quantities listed in table 3.
[c] Iso Clear ® 55% High Fructose Corn Syrup (Cargill Sweeteners, Minneapolis, MN) added to have same total DE as sucrose
[d] milk placed in Roger's Pan Single Effect Evaporator with 26 inches of vacuum at room temperature (no heating) for half an hour
[e] Natural and Artificial Vanilla Flavor Powder Blend NV-31,614 (Robertet, Piscataway, NJ)

Eight milk formulations (Table 17) were chosen to test the following variables: (1) the sweetener high fructose corn syrup was added (HFCS milk) to contrast with sucrose (Control), because high fructose corn syrup is often used in foods and beverages due to its lower cost (Wardlaw, 1999). (2) Flavor was added prior to pasteurization (Control), shortly after pasteurization (Flav Mid), and just before tasting (Flav Lat) to determine the effects of pasteurization and storage on flavor. (3) One quantity of flavor (Control) was added in contrast to twice the quantity (2x Flav) to see if extra flavor has a protective effect on shelf-life. (4) Milk was pasteurized at temperature 89°C (Control) vs. 99°C (High Temp) to see how much raising the temperature impacts flavor. (5) Vanilla flavor (Add Van) was added vs. no vanilla flavor (Control) to continue research from a previous study on the influence of vanilla on flavor and keeping quality. (6) Milk was de-aerated (Vacuum) vs. not de-aerated (Control) to see if reducing oxygen decreases microbial spoilage.

Table 26: Components, their odor descriptions, and their proportions for Strawberry Flavor used in these experiments.

Ingredient	Odor description[a]	% of flavor
Maltol (3-hydroxy-2-methyl-4H-pyran-4-one)	sweet, caramel, cotton candy, fruit, jam, baked bread	2.5% added, extra allowed to precipitate
aldehyde C16 (ethyl methyl phenyl glycidate)	sweet, berry, strawberry, fruity, tutti-frutti and floral nuances	0.5%
cis-3-hexenol	green, foliage, fresh, oily, cut grass	0.25%
Ethyl butyrate	fruity, juicy, fruit, pineapple, cognac	0.5%
Isoamyl isovalerate (3-methylbutyl-3-methyl butyrate)	sweet, fruity, green, ripe, apple, jammy, tropical	0.25%
Ethyl isovalerate (ethyl-3-methyl butyrate)	sweet, fruity, spice, metallic, green, pineapple and apple nuances	0.25%
Ethyl hexanoate	sweet, pineapple, fruity, waxy, banana, green and ester nuances	0.25%
γ-decalactone	fruity, creamy, peach, apricot, syrup and fatty nuances	0.25%
2-methyl butyric acid	pungent acid, Roquefort cheese	0.16%
Propylene glycol		95.09%

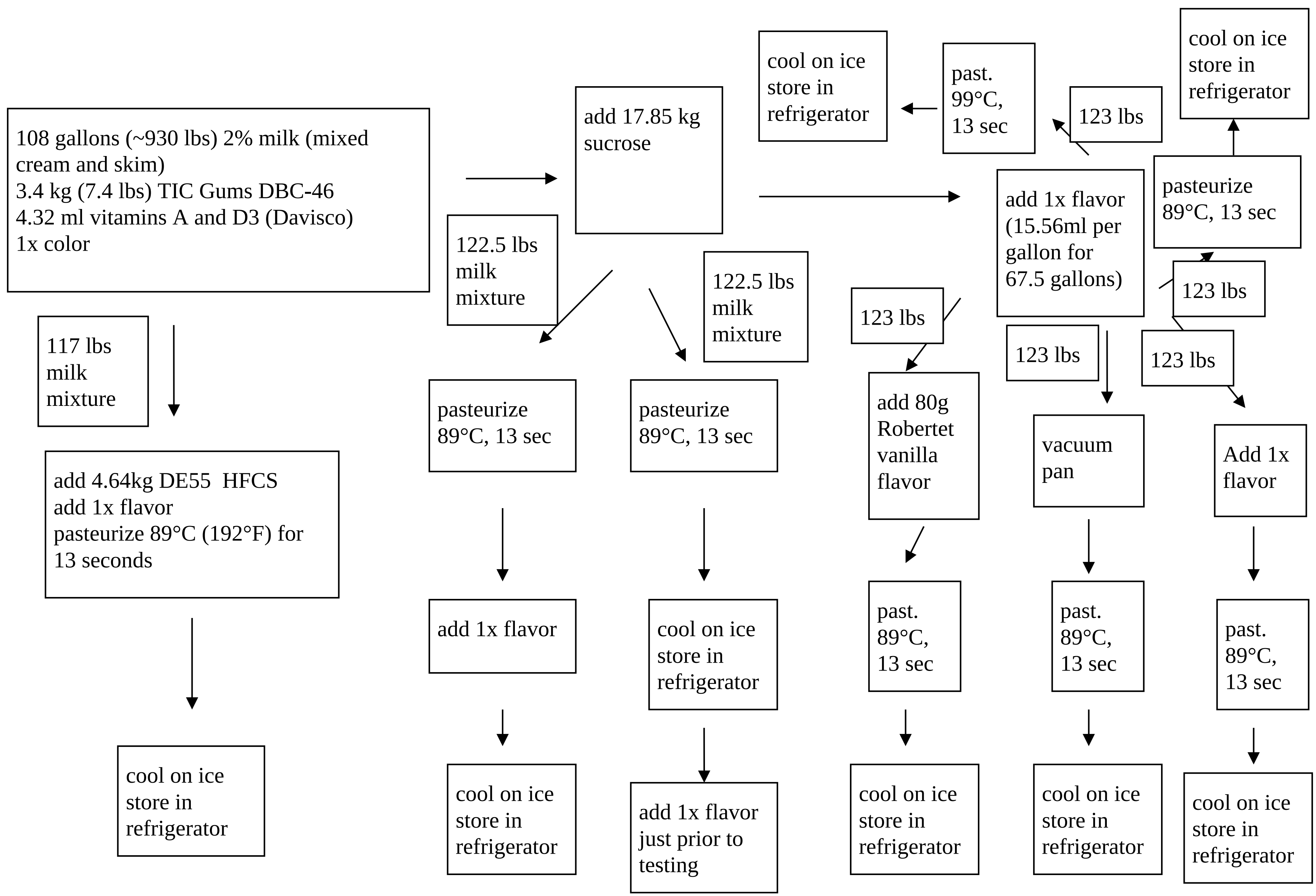
[a] From (http://www.thegoodscentscompany.com)

Sample preparation/Processing

Raw, whole fat, cow's milk was obtained from Dairy Farmers of America and separated into cream and skim milk in the University of Minnesota pilot plant using a Westphalia MP1254 separator. Both cream and skim milk were analyzed by IR for fat content by DQCI Services (Mounds View, MN). Raw skim milk and cream were mixed to reach a final fat level of 2% by weight. Thirteen and a half gallons (116.1 lbs.) of milk were used for each formulation. The raw milk and ingredients (see Table 25) were mixed in a 600 gallon Sunset Milk Cooler with milk being separated in stages as outlined in Diagram 1. Additional ingredients were mixed using a using a Lightenin Mixer (Rotation, Model C4).

The milks were pasteurized (89°C for 13 sec using a Microthermics® UHT/HTST Lab Electric Model 25HV Hybrid), unless otherwise specified, and homogenized (1500psi) using a GEA Niro Soavi homogenizer, Type NS2006H, CP-1, S06H-4796. Then we filled (using a Microthermics® Clean Fill Hood & Sterile Product Outlet) 16oz. opaque HDPE containers (cleaned with 200ppm hypochlorite solution and rinsed with distilled, autoclaved water) with the formulations. The filled bottles were capped and placed on ice to continue cooling the milk (that was still warm from pasteurization). The bottles were then transferred to a large refrigerator (4-7°C) to be stored until analysis. Milks were prepared all in one day and tested weekly. A flow chart of processing steps can be found in Figure 5.

Figure 5: Flow chart showing general mixing, pasteurization, and packaging of strawberry flavored milks utilized for this paper (found on the following page).


108 gallons (~930 lbs) 2% milk (mixed cream and skim)
3.4 kg (7.4 lbs) TIC Gums DBC-46
4.32 ml vitamins A and D3 (Davisco)
1x color
117 lbs milk mixture
add 4.64kg DE55 HFCS
add 1x flavor
pasteurize 89°C (192°F) for 13 seconds
cool on ice store in refrigerator
add 17.85 kg sucrose
122.5 lbs milk mixture
pasteurize 89°C, 13 sec
add 1x flavor
cool on ice store in refrigerator
122.5 lbs milk mixture
pasteurize 89°C, 13 sec
cool on ice store in refrigerator
add 1x flavor just prior to testing
cool on ice store in refrigerator
past. 99°C, 13 sec
123 lbs
add 1x flavor (15.56ml per gallon for 67.5 gallons)
123 lbs
add 80g Robertet vanilla flavor
past. 89°C, 13 sec
cool on ice store in refrigerator
123 lbs
vacuum pan
past. 89°C, 13 sec
cool on ice store in refrigerator
cool on ice store in refrigerator
pasteurize 89°C, 13 sec
123 lbs
123 lbs
Add 1x flavor
past. 89°C, 13 sec
cool on ice store in refrigerator

Microbial Testing

Please refer to Chapter 3 for information on microbial testing.

Descriptive Analysis

Please refer to Chapter 2 for a discussion of a panel developed a lexicon for flavored milk attributes. A second group of panelists, eleven University of Minnesota students (9 females and 2 males, ages 22 to 51) were recruited by Departmental email announcements. The eleven panelists were presented with various stored milk samples in 2 oz. cups. The samples were identified by three digit random number codes. The panelists were asked to rate the samples on 19 attributes. The training consisted of a two one-hour sessions acquainting panelists with references for the 19 attributes and an additional practice session using an unlabeled line scale (15 points in length) on a subset of milk samples. The samples of seven treatments for the study itself were presented to this second panel in duplicate during one session each week. Rating questionnaires were created with SIMS2000 software. Statistical analysis of the sensory studies was calculated using SAS software. Sensory testing was conducted by the Sensory Center at the University of Minnesota (St. Paul, MN).

Gas Chromatography/Solid Phase Microextraction (GC/SPME)

For each stored week of milk, five 4 ml aliquots were taken from two bottles of milk (2 from one bottle and 3 from the other bottle) and poured into separate 20ml vials. Samples were capped with septa and stored in a freezer until the day before analysis when the samples were placed in a refrigerator (4-7°C) to warm up. Samples were run in a balanced incomplete block design with three Weeks per block and ten blocks, so each sample was to be run five times. Each of the Weeks was to be run with every other Week. Samples were randomized within each level using a random number table. However, in the first block, the autosampler malfunctioned (the elastic to retract the fiber became worn out and quit retracting), so the first block of samples was lost along with a number of SPME fibers. Chapter 4 describes the process used to sample and test the milks using GC/MS. Statistics were calculated using SAS software.

Statistical design

This study had a statistical design of a 7x8 factorial for sensory analysis and an 8x8 factorial for instrumental analysis. The study used repeated measures as each milk formulation was mixed and pasteurized on one day and then sampled each week. All milk formulations were compared with the control. A few more contrasts of interest were the comparison of the control milk with milk with strawberry color and milk with strawberry color and flavor added as well as milk with strawberry added prior to pasteurization, just after pasteurization, and just before sampling. The statistical model for sensory evaluation included the Treatment (6 degrees of freedom), the Week (the time stored – 3 degrees of freedom), and the interaction between Treatment and Week. The weeks were a block as all the samples for one week were presented in the same hour, but the samples for other weeks were not presented at the same time. The statistical model for GC/MS included the Treatment (7 degrees of freedom – because Flav Mid was included), the Week (5 degrees of freedom as weeks 0 and 5 were also tested), and the interaction between Treatment and Week. Each block varied, but it consisted of a combination of samples from three weeks.

Results and Discussion

Descriptive Analysis

Ten panelists tasted and rated milk formulations on nineteen sensory attributes over a period of four weeks. Statistical comparison of changes in milk attributes by milk formulation and time and the interaction of those two variables can be found in Table 27. Attributes with significant treatment effects are compared in Table 28. Attributes with significant storage time effects are compared in Table 29. Attributes with interactions between time and formulation are shown in Figures 6 (sweet), 7 (sour), 8 (bitter), 9 (oxidized), 10 (dirty), and 11 (other off flavors).

Sweet intensity remained constant in 2x Flav, Flav Lat, and HFCS, while it decreased in other milks (Figure 6). High fructose corn syrup was added to milk in quantities to equal the dextrose equivalents of sucrose. However, the quantity of high fructose corn syrup added was greater than the quantity of sucrose added, so HFCS contained a greater proportion of sweetener than the milks sweetened with sucrose. 2x Flav and Flav Lat were also both sweeter than the Control. Flav Lat had the flavor added just prior to tasting, so the pasteurized milk was stored separately from the strawberry flavor. 2x Flav had more flavor than Control, while Flav Lat may have had a fresher flavor than Control. Flavor ingredients with sweet components such as maltol, ethyl methyl phenyl glycidate, or isoamyl isovalerate may have been more prevalent in these two samples and contributed to the perceived sweetness. Lavin and Lawless (1998) found that adding vanillin made milk seem sweeter, and the extra or fresher strawberry flavor may have had the same effect. The same milks that did not decrease in sweet notes did not increase in sour notes (Figure 7). This could be due to mixture suppression in that the sour notes masked the sweet notes in the milks with greater sour notes, while the sweetness remained evident in the milks without sour notes (McBurney and Bartoshuk, 1973). Salty notes, most likely originating from the salt in the gum mixture, decreased with time (Table 29). Salt is a major ingredient in the thickener, which was created for chocolate milk. In previous studies (Strohman – Chapters 2, 3, and 4), salty notes did not significantly change with time. In this study, the salty decreases mimic the decreases in malty notes. Perhaps panelists associated one with the other.

We removed air from Vacuum milk to determine whether this would reduce the growth of aerobic bacteria. Two differences were noted. Bitter and oxidized notes increased in Vacuum, but not in the other milks (Figures 8 and 9). Many articles have found bitter flavor to originate from either lipolysis or from microorganisms (Shipe, 1978). Although the milk was partially de-aerated, once packaged the bottles physically bulged with diffused air. Aerobic Plate Counts were consistently higher in Vacuum milk than in other milk formulations.

Table 27: Significant differences (at α<0.05) in attribute intensity ratings of strawberry flavored milk by descriptive analysis panel.

Attribute	Treatment [a]	Week [b]	Treatment*Week [c]
Sweet	<0.0001	<0.0001	**0.0027**
Sour	<0.0001	<0.0001	**<0.0001**
Salty	NS	**<0.0001**	NS
Bitter	NS	<0.0001	**<0.0001**
Umami	NS	NS	NS
Malty	**0.0011**	**0.0018**	NS
Butter/diacetyl	NS	**0.0008**	NS
Cream/fresh dairy	NS	**<0.0001**	NS
Cooked/caramel	**<0.0001**	**<0.0001**	NS
Fruity	**<0.0001**	**<0.0001**	NS
Strawberry	**<0.0001**	**<0.0001**	NS
Vanilla	**<0.0001**	**0.0383**	NS
Green	**0.0296**	NS	NS
Oxidized/cardboardy	0.0231	<0.0001	**0.0013**
Sulfur/eggy	**0.0053**	**<0.0001**	NS
Dirty/smoky/etc.	<0.0001	<0.0001	**0.0002**
Chemical/medicinal	**0.0462**	**<0.0001**	NS
Lactones/coconut	NS	**0.0182**	NS
Other off flavors	<0.0001	<0.0001	**<0.0001**

ANOVA comparison of mean peak areas for treatment (milk formulation), week (time stored), and treatment*week (interactions).

* values in **bold** will be discussed; attributes in *italics* have significant interactions (milk*week); attributes in **bold** are significant by week or treatment with no significant interactions.

[a] Treatment refers to differences between different milk formulations. More information on these findings is in Table 28. Treatment degrees of freedom = 6

[b] Weeks refers to amount of time the milk was stored. More information on these findings is in Table 29. Week degrees of freedom = 3

[c] Treatment*Weeks refers to interactions. More information on these findings is in figures 6-11. Treatment*Week degrees of freedom = 18

Table 28: The influence of milk formulation on sensory attribute intensity.

Attribute	HFCS	Vacuum	HighTemp	AddVan	Control	2x Flav	FlavLat
Malty	0.9[a]	0.9[a]	1.0[a]	1.3[b]	0.9[a]	1.1[a]	1.0[a]
Cooked/ caramel	1.1[a]	0.9[a]	1.2[a]	1.5[b]	1.0[a]	1.0[a]	1.0[a]
Fruity	3.5[b]	3.1[b]	2.9[b]	2.2[a]	3.1[b]	4.3[c]	3.4[b]
Strawberry	3.1[c,d]	2.7[b]	2.7[b]	2.0[a]	2.7[b]	4.3[d]	3.4[c]
Vanilla	0.8[a]	0.5[a]	0.7[a]	3.7[b]	0.6[a]	0.6[a]	0.7[a]
Green	0.1[a,b]	0.1[a,b]	0.1[a]	0.1[a]	0.1[a,b]	0.2[b]	0.1[b]
Chemical/ medicinal	0.3[a,b]	0.4[a,b]	0.3[a,b]	0.4[a,b]	0.4[a,b]	0.4[b]	0.2[a]

Mean ratings by an 11 person trained panel. All ratings are on an unlabeled line scale (15 points in length).

[a-e] Different letters denote differences in mean ratings within rows (attributes) using the Student-Newman-Keuls Test within the method of least squares (α=0.05, error degrees of freedom=66). It has been assumed that this is a general linear model.

*The sulfur/eggy attribute was found to be significantly different between treatments in ANOVA but not in Student-Newman-Keuls

Table 29: The influence of storage time on various sensory attributes on all formulations together

Attribute	Stored 1 Week	Stored 2 Weeks	Stored 3 Weeks	Stored 4 Weeks
Salty	1.5[c]	1.2[b]	0.7[a]	0.8[a]
Malty	1.4[c]	1.2[b]	0.7[a]	0.6[a]
Butter/diacetyl	1.2[c]	1.2[c]	0.6[b]	0.4[a]
Cream/fresh dairy	2.1[b]	2.0[b]	1.2[a]	1.1[a]
Cooked/caramel	1.6[c]	1.2[b]	0.9[a]	0.7[a]
Fruity	3.8[c]	3.4[b]	3.1[b]	2.5[a]
Strawberry	3.6[c]	3.2[c]	2.8[b]	2.3[a]
Vanilla	1.1[a]	1.4[b]	0.9[a]	0.8[a]
Sulfur/eggy	0.1[a]	0.1[a]	0.3[b]	0.4[b]
Chemical/ medicinal	0.3[b]	0.2[a]	0.4[b]	0.5[c]
Lactones/ coconut	0.4[b]	0.6[c]	0.2[a]	0.2[a]

Mean ratings by an 11 person trained panel. All ratings are on an unlabeled line scale (15 points in length).
[a-c] Different letters denote differences in mean ratings within rows (attributes) using the Student-Newman-Keuls Test within the method of least squares (α=0.05, error degrees of freedom=66). It has been assumed that this is a general linear model

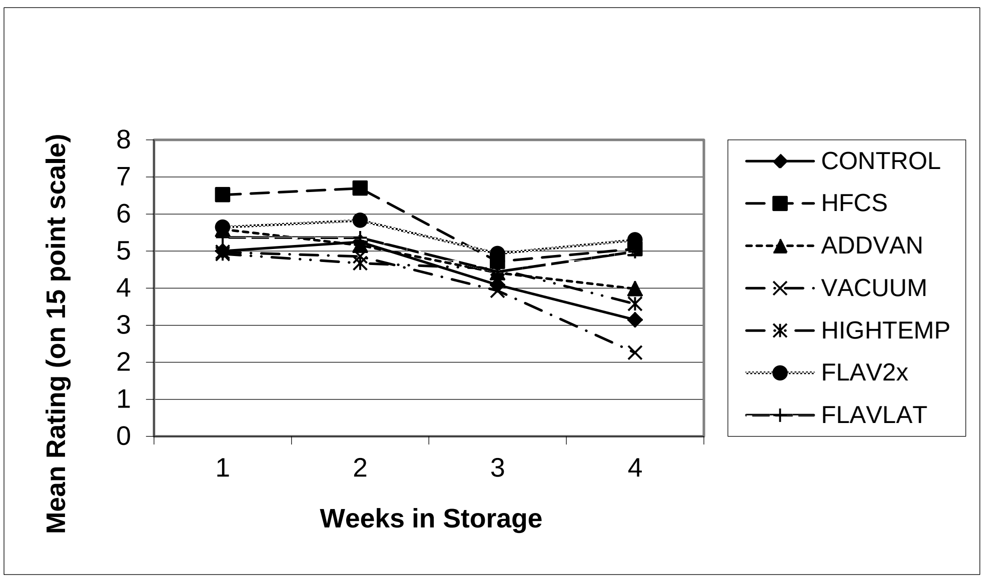


Figure 6: The influence of milk formulation on mean sweet attribute ratings in strawberry flavored milk over time.

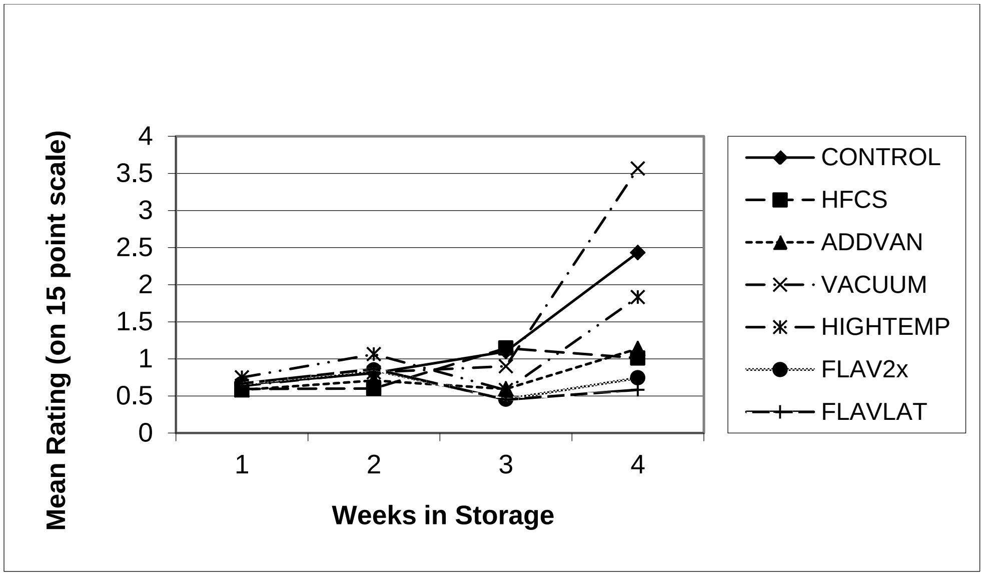


Figure 7: The influence of milk formulation on mean sour attribute ratings in strawberry flavored milk over time.

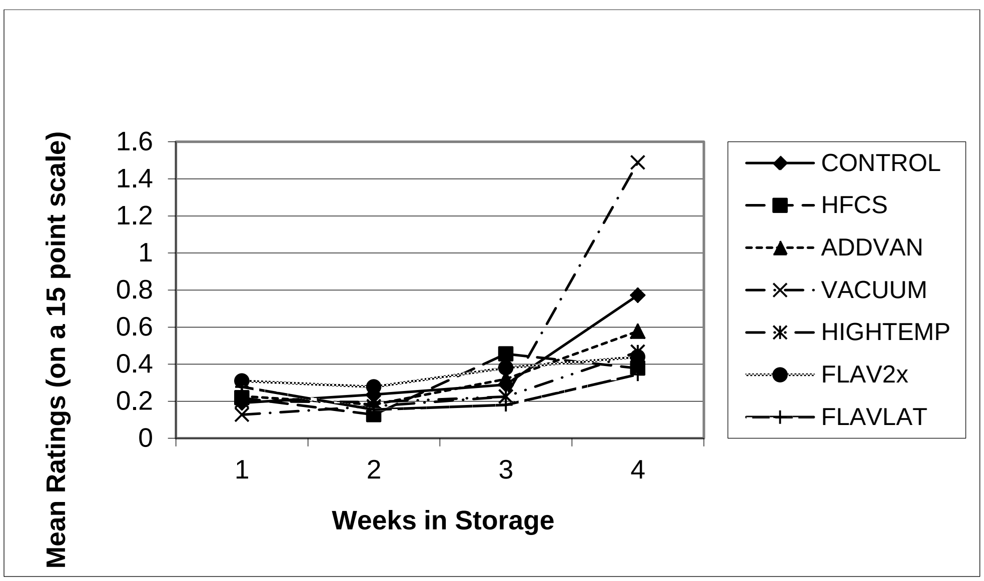


Figure 8: The influence of milk formulation on mean bitter attribute ratings in strawberry flavored milk over time.

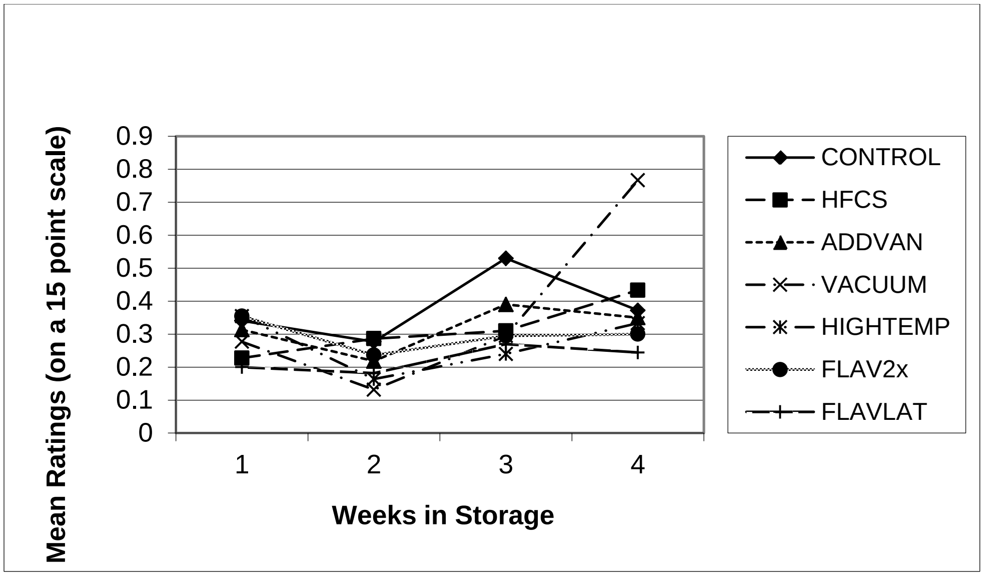


Figure 9: The influence of milk formulation on mean oxidized attribute ratings in strawberry flavored milk over time.

Malty, cooked/caramel, and vanilla notes were of greater intensity in Add Van than in the other milks and malty, cooked/caramel, and vanilla notes decreased with time overall (Tables 28 and 29). This may indicate that the malty and cooked/caramel notes were associated with the vanilla flavor itself. Strohman (Chapter 3) found that vanilla flavored milk decreased in malty, cooked/caramel, and vanilla notes with time, while milks without vanilla flavor did not change in these attributes. Cooked flavors often originate as sulfhydryls from the breakdown of β-lactoglobulin during pasteurization (Hutton and Patton, 1952). These cooked flavors typically decrease within a few days of the heat treatment (Hutton and Patton, 1952; Shipe et al., 1978). Butter/diacetyl and cream/fresh dairy notes decreased with time (Table 29). These are fresh milk flavors and are associated with pleasant flavors, so they may decrease with time as the milk flavor becomes less pleasant. Strohman (Chapter 3) found that cream/fresh dairy notes decreased with time in all of the flavored milks. Strohman found that butter/diacetyl notes decreased significantly in vanilla flavored milk (Chapter 3). Strohman also found that cream/fresh dairy and butter/diacetyl notes were masked by strawberry flavoring (Chapter 2).

Fruity notes were greater in intensity in 2x Flav than Control and lower in intensity in Add Van than Control (Table 28). Since 2x Flav had more strawberry flavor than Control, and strawberry flavor can be considered fruity, it follows that 2x Flav would be more fruity. Add Van contained vanilla flavor and this may have masked the fruity notes. Fruity flavor also decreased with time in all milks (Table 29). Strohman (Chapters 3 and 4) did not find that fruity notes changed significantly with time in strawberry flavored milks. However, the pink color of the milk was visible only for weeks one and two, so the disappearance of pink color may have influenced the decrease in intensity of fruity ratings. Strawberry notes were greatest in 2x Flav, second greatest in Flav Lat, next in Control and most other milks, and least in Add Van (Table 28). Since 2x Flav contained more strawberry flavor than the other milks, it is to be expected that strawberry notes would be greatest in intensity in this formulation. In Flav Lat, the milk was stored separately from the flavor (since flavor was added just prior to tasting). Since Flav Lat was greater in intensity in strawberry notes than Control, this suggests reactions between the flavor and the milk decreased the available strawberry flavor. Add Van probably had its strawberry notes masked by the added vanilla flavor. Strawberry notes also decreased with time (Table 29). Strohman (Chapter 3) did not find that strawberry notes changed significantly with time in strawberry flavored milks, however, strawberry notes did decrease over time in a later study (Chapter 4). Strohman also found a decrease of strawberry notes in concert with an increase in sour and other off notes (Chapter 4).

Green, oxidized/cardboardy, sulfur/eggy, and chemical/medicinal notes all had an intensity rating of 0.5 or below on a 15 point scale, so there is not enough information to discuss the changes in these attributes even when the changes are significant. Dirty/smoky/etc. increased with storage time in Vacuum and Add Van (Figure 10). Other off flavors also increased in Vacuum (Figure 11). The dirty/smoky/etc. and other off flavors in Vacuum were likely caused by oxidation related to a sharp increase in microorganisms in Vacuum at week 4. The original dirty/smoky/etc. flavor in the vanilla flavored milk probably was a product of the vanillin, but it may have increased due to oxidation as well. Lactone/coconut notes first increased and then decreased with time (Table 29). Badings (1991) tentatively proposed that compounds with similar flavor are produced by esterification of 4- and 5- hydroxy fatty acids when heated, so lactones/coconut notes may be a result of the pasteurization process.

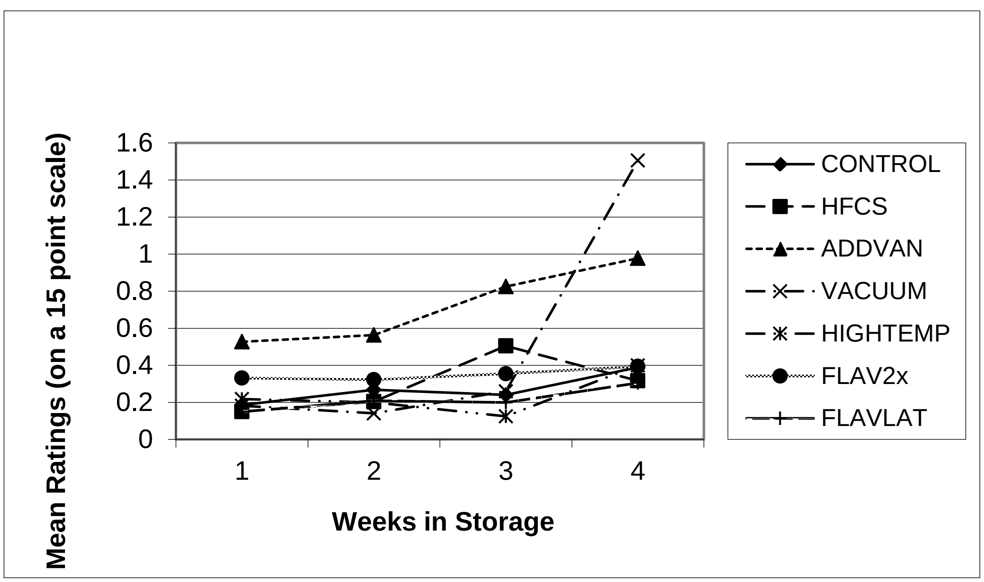


Figure 10: The influence of milk formulation on mean dirty attribute ratings in strawberry flavored milk over time.

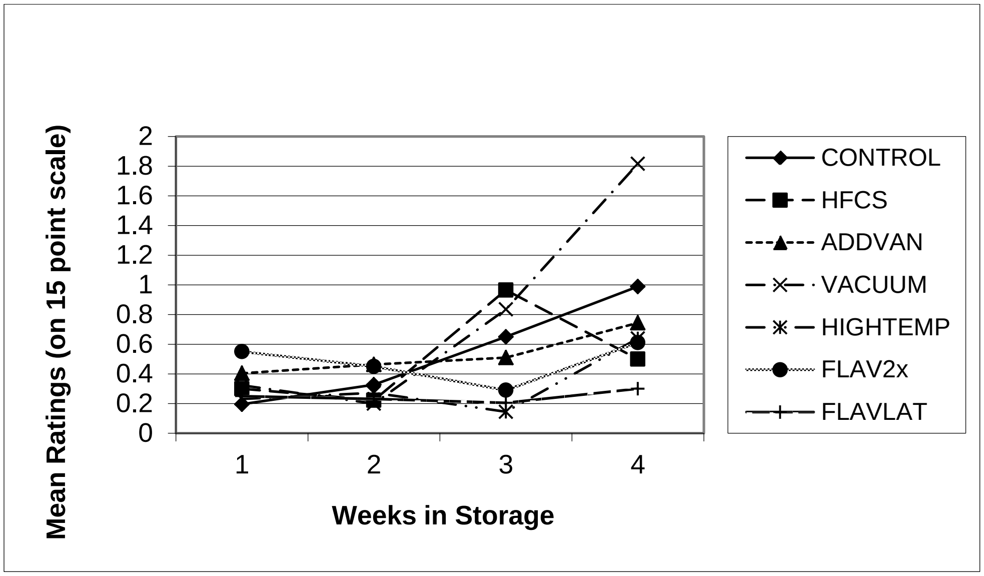


Figure 11: The influence of milk formulation on mean other off flavor attribute ratings in strawberry flavored milk over time.

GC/SPME

For GC/MS, the statistical design used was balanced incomplete blocks, but during the actual analysis, the first block was lost due to mechanical problems. All samples from one week were analyzed together, but not all weeks were analyzed with every other week due to the first block being lost. Half of the milk treatment/time varieties were analyzed 5 times and half were analyzed 4 times due to the first block being lost. Most blocks showed significant variation from the other blocks in measurements of the compounds tentatively identified (Table 30). Information about treatment variation can be found in Table 31. Information about time variation is in Table 32. Interactions are presented in figures 12 (2-butanone), 13 (ethyl butyrate), 14 (*cis*-3-hexenol), and 15 (hexanoic acid).

Table 30: Significant differences in tentatively identified compounds in strawberry flavored and/or colored milk by mass spectrometry (degrees of freedom, F value, p value)

Compound*	Block	Treatment [a]	Weeks [b]	Treatment* Weeks [c]
acetone	8, 16.05, <0.001	**7, 21.07, <0.001**	**5, 13,38, <0.001**	35, 1.04, 0.4208
2-butanone	8, 16.55, <0.001	7, 5.39, <0.001	5, 3.74, 0.0032	**35, 1.60, 0.0272**
ethyl butyrate +	8, 85.39, <0.001	7, 85.56, <0.001	5, 1.90, 0.0968	**35, 1.63, 0.0235**
ethyl-3-methyl butyrate +	8, 40.71, <0.001	**7, 64.89, <0.001**	5, 0.29, 0.9200	35, 0.86, 0.6916
ethyl hexanoate +	8, 2.36, 0.0199	**7, 13.62, <0.001**	5, 1.96, 0.0880	35, 1.44, 0.0674
1-hydroxy-2-propanone (log)	8, 7.27, <0.001	7, 1.84, 0.0848	**5, 14.61, <0.001**	35, 0.97, 0.5189
3-methyl-3-methylbutyl butyric acid +	8, 15.82, <0.001	**7, 124.10, <0.001**	5, 1.31, 0.2638	35, 1.00, 0.4804
cis-*3-hexenol +*	8, 26.44, <0.001	7, 90.20, <0.001	5, 7.06, <0.001	**35, 1.85, 0.0058**
butyric acid	8, 1.12, 0.3527	7, 0.95, 0.4682	5, 1.48, 0.2019	34, 1.12, 0.3150
2-methyl butyric acid (log) +	8, 8.52, <0.001	**7, 7.77, <0.001**	**5, 10.73, <0.001**	35, 1.00, 0.4744
hexanoic acid (log)	8, 18.22, <0.001	7, 5.12, <0.001	5, 71.36, <0.001	**35, 2.67, <0.001**
ethyl methyl phenyl glycidate +	8, 8.85, <0.001	**7, 97.06, <0.001**	5, 2.09, 0.0692	35, 1.37, 0.0978
γ-decalactone+	8, 16.51, <0.001	**7, 44.64, <0.001**	**5, 4.29, 0.0011**	35, 1.06, 0.3903

significant differences calculated with PROC GLM (type III SS) with means adjusted for blocks. + compound is part of original flavor * values in **bold** will be discussed; attributes in *italics* have significant interactions (milk*week); attributes in **bold** are significant by week or treatment with no significant interactions

[a] Treatment refers to differences between different milk formulations. More information on these findings is in Table 29.

[b] Weeks refers to amount of time the milk was stored. More information on these findings is in Table 32.

[c] Treatment*Weeks refers to interactions. More information on these findings is in figures 12-15.

GC/SPME analysis was conducted on the stored milk samples to see if instrumental analysis could correlate quantities of compounds with sensory attribute intensities. SPME fibers are sensitive enough to pick up compounds at the low ppb range, but can be overwhelmed if large amounts of compounds are present. Although GC techniques are objective and may measure compounds down to a 10^{-13} concentration, there is no assurance that they will measure compounds that are important to determining the liking of the milk. Despite this, attempting to measure compounds is still important for purposes of our study, because identifying compounds related to sensory descriptions can aid in corroborating our identifications of reasons for flavor changes.

In Table 29 we observed that 2x Flav has less of two compounds (acetone and 2-butanone) and more of eight compounds (ethyl butyrate, 3-methyl-3-butyl-butyric acid, *cis*-3-hexenol, ethyl methyl phenyl glycidate, γ -decalactone, ethyl-3-methyl butyrate, ethyl hexanoate, and 2-methyl butyric acid) than the Control does. Acetone and butanone can both be transferred to milk by feed consumed by the cow shortly before milking (and in humans from normal metabolism) (Badings, 1991). These compounds may be lesser in 2x Flav, because of the abundance of strawberry flavor compounds and our sampling method. SPME fibers pick up only so many aroma compounds and stronger flavor compounds could out-compete regular metabolism compounds such as acetone. All eight of the compounds in which 2x Flav is greater than Control are compounds found in the strawberry flavor.

Table 31: Influence of milk formulation on peak areas of tentatively identified compounds in strawberry flavored milk *

Compound	HFCS	Vacuum	High Temp	Add Van	Control	2xFlav	Flav Lat	Flav Mid
acetone	$6.0x10^7$ b	$6.5x10^7$ b	$6.5x10^7$ b	$6.9x10^7$ b	$6.6x10^7$ b	$3.6x10^7$ a	$6.9x10^7$ b	$6.2x10^7$ b
ethyl-3-methyl butyrate +	$2.1x10^9$ c	$2.0x10^9$ b,c	$2.0x10^9$ c	$1.8x10^9$ a,b,c	$2.0x10^9$ c	$3.3x10^9$ d	$1.7x10^9$ a,b	$1.6x10^9$ a
ethyl hexanoate +	$1.4x10^9$ b,c	$8.5x10^8$ a,b	$7.9x10^8$ a	$8.4x10^8$ a,b	$8.1x10^8$ a	$1.8x10^9$ c	$7.9x10^8$ a	$5.4x10^8$ a
3-methyl-3-methylbutyl butyric acid +	$3.6x10^8$ c	$3.6x10^8$ c	$3.7x10^8$ c	$3.2x10^8$ b,c	$3.4x10^8$ b,c	$7.6x10^8$ d	$2.8x10^8$ a,b	$2.3x10^8$ a
2-methyl butyric acid (log)+	$1.3x10^7$ c,d	$8.9x10^6$ a,b,c	$6.9x10^6$ a,b,c	$7.6x10^6$ a,b,c	$8.5x10^6$ b,c,d	$1.4x10^7$ d	$5.5x10^6$ a	$6.4x10^6$ a,b
ethyl methyl + phenyl glycidate	$9.2x10^6$ b	$8.3x10^6$ b	$8.2x10^6$ b	$9.1x10^6$ b	$8.7x10^6$ b	$1.7x10^7$ c	$7.6x10^6$ a,b	$6.7x10^6$ a
γ-decalactone +	$6.5x10^6$ b	$5.9x10^6$ b	$5.8x10^6$ a,b	$5.8x10^6$ a,b	$6.2x10^6$ b	$1.1x10^7$ c	$5.4x10^6$ a,b	$4.5x10^6$ a

+ compound is part of original flavor
* Significant differences in TukeyHSD of compounds tentatively identified through GC/MS/SPME.

Add Van, High Temp, and Vacuum were not different than Control. HFCS is greater than Control in one compound (ethyl hexanoate) (Table 31). Flav Mid is less than Control in six compounds (ethyl butyrate, 3-methyl-3-butyl-butyric acid, *cis*-3-hexenol, ethyl methyl phenyl glycidate, γ -decalactone, ethyl-3-methyl butyrate) (Table 31). All six of these compounds are found in the strawberry flavor. Since Flav Mid was not homogenized with the flavor in it, the strawberry flavor may have reacted differently in the milk over time. Flav Lat is less than Control in two compounds (ethyl-3-methyl-butyrate, 2-methyl butyric acid) (Table 31). In the Control, these two compounds may have increased due to an interaction with the milk during the storage time.

Acetone, 2-butanone, and *cis*-3-hexenol and γ -decalactone fluctuated with storage time, but did not trend to increase or decrease (Table 32). Butanone is relatively stable in milk with time (Jaddou et al., 1978). *Cis*-3-hexenol and γ -decalactone were part of the strawberry flavor. 1-hydroxy-2-propanone, 2-methyl-butyric acid, hexanoic acid increased with time (Table 32). 1-hydroxy-2-propanone may be formed by dehydration of glycerol during Maillard reactions (Cerny and Guntz-Dubini, 2006). This may have caused the increase in 1-hydroxy-2-propanone. 2-butanone, ethyl butyrate, cis-3-hexenol, and hexanoic acid had interactions (Figures 12, 13, 14, and 15). Hexanoic acid peak areas for Flav Lat did not increase sharply at week three along with the other milks (Figure 15). Most milk formulations increased in hexanoic acid with time, so storing the flavor separately from the milk may have decreased oxidation leading to hexanoic acid production. We had hoped for more solid information from the interactions, but what we found was more erratic.

Table 32: Influence of time on peak areas of tentatively identified compounds in strawberry flavored milk *

Compound	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5
acetone	6.1 $x10^{7\ a,b}$	7.6 $x10^{7\ c}$	6.4 $x10^{7\ b}$	5.3 $x10^{7\ a}$	6.1 $x10^{7\ a,b}$	5.5 $x10^{7\ a,b}$
1-hydroxy-2-propanone (log)	5.8 $x10^{7\ a}$	7.6 $x10^{7\ b}$	1.3 $x10^{8\ b}$	1.8 $x10^{8\ c}$	9.9 $x10^{7\ c}$	1.1 $x10^{8\ c}$
2-methyl butyric acid (log) +	4.8 $x10^{6\ a}$	1.0 $x10^{7\ a,b}$	1.3 $x10^{7\ b,c}$	5.9 $x10^{6\ a}$	8.2 $x10^{6}$ a,b,c	1.1 $x10^{7\ c}$
γ-decalactone +	6.6 $x10^{6\ a,b}$	5.6 $x10^{6\ a}$	6.0 $x10^{6\ a}$	6.2 $x10^{6\ a,b}$	7.3 $x10^{6\ b}$	6.6 $x10^{6\ a,b}$

+ compound is part of original flavor
* Significant differences in TukeyHSD of compounds tentatively identified through GC/MS/SPME.

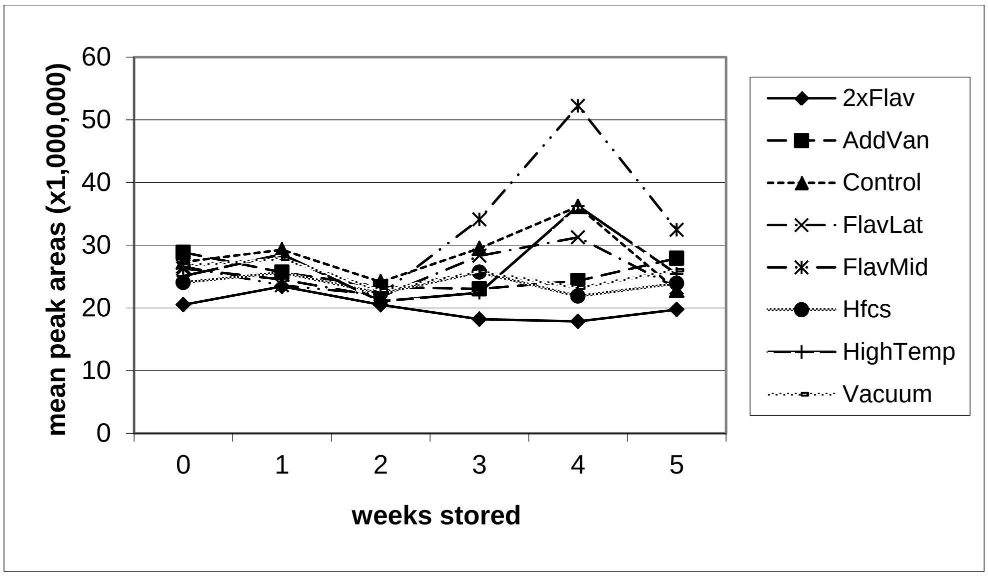


Figure 12: Influence of milk formulation on 2-butanone concentration changes over time in strawberry flavored milk.

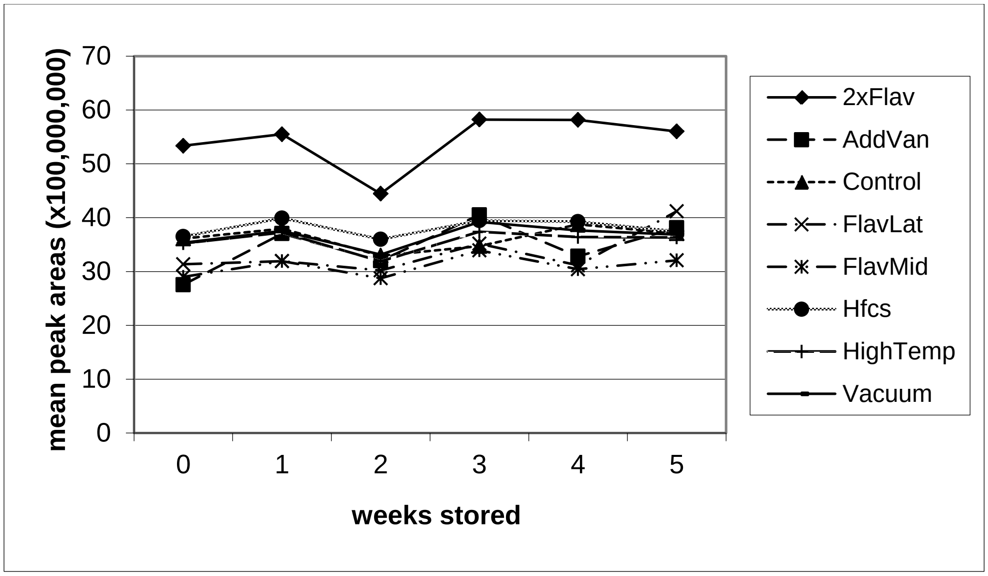


Figure 13: Influence of milk formulation on ethyl butyrate concentration changes over time in strawberry flavored milk.

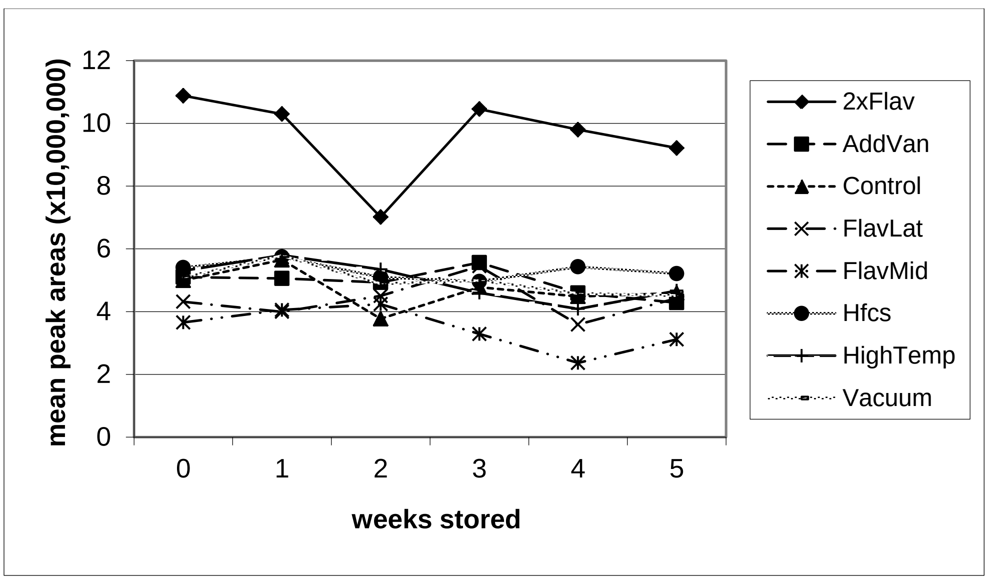


Figure 14: Influence of milk formulation on *cis*-3-hexenol concentration changes over time in strawberry flavored milk.

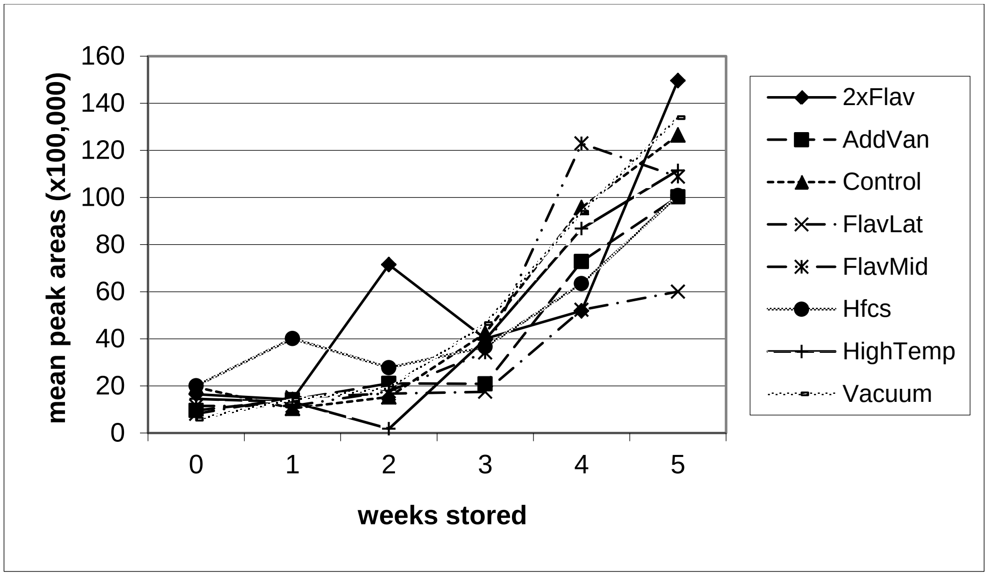


Figure 15: Influence of milk formulation on hexanoic acid concentration changes over time in strawberry flavored milk.

Bitter ratings correlated positively with the log of hexanoic acid peak areas (Table 33). Hexanoic acid flavor has been described as mildly sour, fatty, bitter, goaty, and sweaty (http://www.thegoodscentscompany.com). In addition, hexanoic acid correlated with a majority of the unpleasant flavors. This indicates that hexanoic acid increased as unpleasant flavors increased. Panelists increased their ratings of unpleasant attributes with time and decreased their ratings of pleasant attributes with time. Hexanoic acid flavor does not match all of the flavor attributes, so some of the correlation may be spurious. Green attribute ratings correlated positively with most compounds in the original strawberry flavor (Table 33). However, green attribute ratings were generally low (0.1 and 0.2).

Table 33: Correlations between sensory attributes ratings and GC/MS peak areas for specified compounds.* (Change in attributes explained by change in compounds.)

Attribute	Compound (correlation) direction
Sweet	ethyl hexanoate (0.25)+
Sour	(log) hexanoic acid (0.40)+
Salty	acetone (0.25)-; (log) hexanoic acid (0.42)-; (log) 1-hydroxy-2-propanone (0.28)-
Bitter	**(log) hexanoic acid (0.64)+**
Umami	2-butanone (0.31)+; ethyl hexanoate (0.35)-
Malty	(log) hexanoic acid (0.52)-
Butter/diacetyl	(log) hexanoic acid (0.59)-
Cream/fresh dairy	(log) hexanoic acid (0.53)-
Cooked/caramel	(log) hexanoic acid (0.48)-
Fruity	*cis* 3 hexenol (0.49)+; ethyl-3-methyl butyrate (0.40)+; ethyl butyrate (0.47)+; ethyl hexanoate (0.39)+; ethyl methyl phenyl glycidate (0.29)+; (log) 2-methyl butyric acid (0.28)+; 3-methyl-3-methylbutyl butyric acid (0.42)+
Strawberry	*cis* 3 hexenol (0.50)+; ethyl-3-methyl butyrate (0.39)+; ethyl butyrate (0.45)+; ethyl hexanoate (0.31)+; ethyl methyl phenyl glycidate (0.30)+; 3-methyl-3-methylbutyl butyric acid (0.42)+
Vanilla	No compounds had a moderate or strong correlation with this attribute.
Green	acetone (0.60)-; ***cis*-3-hexenol (0.64)+**; **ethyl-3-methyl butyrate (0.70)+**; **ethyl butyrate (0.72)+**; **ethyl hexanoate (0.66)+**; **ethyl methyl phenyl glycidate (0.69)+**; **γ-decalactone (0.72)+**; (log) 1-hydroxy-2-propanone (0.31)+; (log) 2-methyl butyric acid (0.28)+; **3-methyl-3-methylbutyl butyric acid (0.68)+**
Oxidized/cardboardy	(log) hexanoic acid (0.29)+
Sulfur/eggy	(log) hexanoic acid (0.42)+
Dirty/smoky/etc.	No compounds had a moderate or strong correlation with this attribute.
Chemical/medicinal	ethyl methyl phenyl glycidate (0.26)+; γ-decalactone (0.35)+; (log) hexanoic acid (0.45)+
Lactones/coconut	(log) hexanoic acid (0.30)-
Other off flavors	(log) hexanoic acid (0.51)+

* Pearson correlation coefficient (r) can be used to show if two things vary similarly. An r of .8 to 1 (positive or negative) indicates a strong correlation. An r of .5 to .8 (positive or negative) indicates a moderate correlation. (Devore and Peck, 2001) This table shows r-squared values greater than or equal to .25 (.5 x .5) with r-squared values greater than or equal to .64 (.8 x .8) in bold.

The aerobic plate count for Vacuum milk was much higher than for the other milks at week 4 (Figure 16). Flav Lat had the lowest micro count on week 4, while 2x Flav and Add Van also had low counts at week 4. More research can be conducted to determine if adding vanillin or extra strawberry flavor impact reduces microbial counts to, therefore, extend shelf-life of flavored milk.

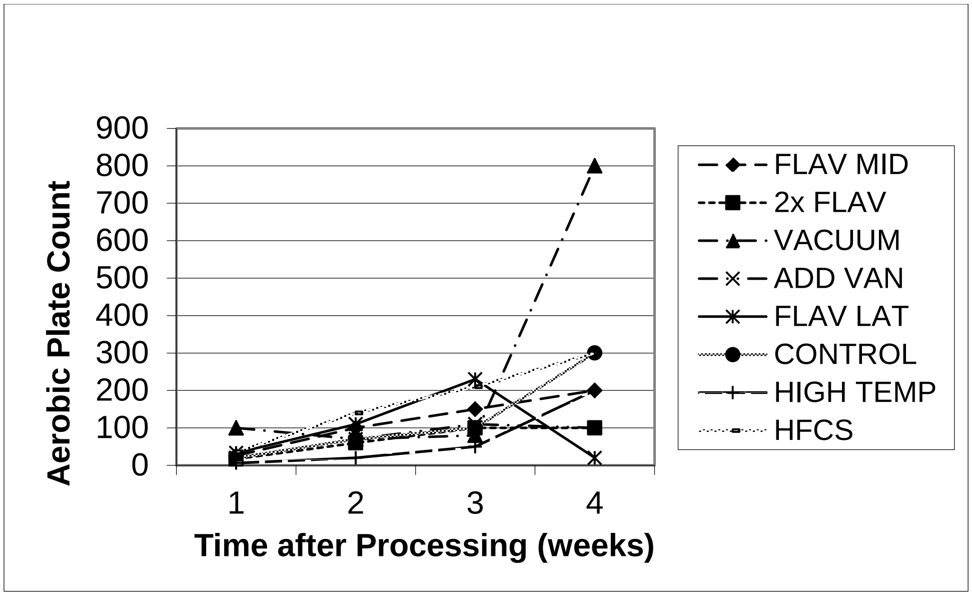


Figure 16: Influence of formulation on changes of aerobic plate count over time in strawberry flavored milk.

Conclusions

In instrumental analysis, Flav Mid was lower in six strawberry flavor compounds than the Control, but since the panel did not taste this formulation, it can not be compared to sensory findings. Since Flav Lat and Flav Mid both had flavor added after pasteurization and Flav Lat contained more flavor than Flav Mid at testing time, flavor interactions with milk during storage decreases the amount of flavor. We expected that milk with flavor added just prior to tasting (Flav Lat) would be greater in flavor than the Control, and it was rated as sweeter, having more strawberry notes, and having fewer other off-flavor notes even though Flav Lat was lower in two strawberry flavor compounds than the Control. This provides an opportunity for future research about adding flavor at later times.

Our hypothesis was correct that flavor deterioration was less in HFCS and 2x Flav milk. HFCS milk was both sweeter and less sour than the Control, but it required the addition of more sweetener than sucrose. 2x Flav was sweeter, less sour, and had more strawberry and fruity notes than the Control. Therefore, adding more strawberry flavor may have a positive impact.

Add Van did sustain itself longer than the Control, but Vacuum faired more poorly. Add Van was less sour, lower in strawberry flavors, and higher in vanilla related flavors than Control. Adding vanilla flavor had too much of an impact on decreasing perceived strawberry flavor to be considered viable. Vacuum had more other off-flavors than the Control did and this seemed to be due more to microorganism growth than to anything else. Therefore, we conclude that de-aerating milk will not provide a benefit to sellers or consumers.

Future Research

Research on the addition of extra flavor or the timing of the addition of flavor to strawberry flavored milk will prove to be valuable.

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Chapter VI:
SUMMARY AND CONCLUSIONS

Hypothesis 1

Strawberry flavored milk becomes more undesirable with time due to the degradation of strawberry flavor (decrease of "good" flavors).

Chapter 2 defined this hypothesis as sweet taste and strawberry flavor being desirable flavors. In Chapter 3, our sensory ratings for strawberry flavor did decrease with time, while the sweet taste ratings varied significantly, but did not trend toward either an increase or a decrease. In Chapter 4, our sensory ratings for strawberry decreased with time, but the instrumental analysis revealed that most strawberry flavor compounds did not change. In Chapter 5, ratings of strawberry intensity decreased with time, but peak areas of strawberry flavor compounds did not. In Chapters 4 and 5, strawberry sensory intensity decreased in concert with sweet intensity ratings, suggesting the two are related. Also, in Chapter 5, milk with flavor added just prior to tasting was rated as being both sweeter and greater in intensity of strawberry notes than was the control. This suggests that the quality of the strawberry flavor may change with time. Therefore, from our research, we conclude that the strawberry flavor itself does not decrease (according to instrumental results), but that people perceive the strawberry flavor to decrease, possibly due to the formation of off flavors.

Hypothesis 2

Strawberry flavored milk becomes more undesirable with time due to the degradation of major milk constituents (increase of "bad" flavors).

In Chapter 2, the attributes that were most affected by the milk itself were sour, bitter, oxidized/cardboardy, dirty/smoky, and other off flavors, which can be generally categorized as unpleasant flavors. These attributes increased with time in at least three out of five of the unflavored milks and had an overall mean rating of at least one in strawberry flavored milk. Therefore, these attributes were used to test this hypothesis. Chapter 3 did find increases in undesirable notes in some milk formulation, but mostly in milks that were unflavored. In Chapter 4, sour, bitter, dirty/smoky, and other off-flavor notes increased with time in the sensory study, while acetic acid and butyric acid both increased with time in general. In Chapter 5, sour, bitter, dirty/smoky, and other off-flavor notes increased with time. In addition, hexanoic acid peak areas increased with time and correlated significantly with most of the undesirable flavor attributes, especially with bitter. Overall, the increase of undesirable flavors over time contributed negatively to the flavor of the milks, which leads us to conclude that our hypothesis is correct.

Hypothesis 3

Strawberry flavor is important in the liking of strawberry milk (e.g. not just sweetness and color).

In Chapter 3, we compared strawberry colored milk with strawberry flavored milk. Strawberry colored milk was rated as lesser in intensity of sweet notes as well as in strawberry notes and greater in intensity of sour, sulfur/eggy, and other off-flavor notes than strawberry flavored milk. This indicates that strawberry flavor masks some undesirable notes. In Chapter 4, strawberry colored milk was again rated as lesser in intensity of sweet notes and strawberry notes than strawberry flavored milk when stored for a short amount of time (one or two weeks). Children rated the strawberry flavor higher in the strawberry flavored milk and they liked the strawberry flavored milk more than the strawberry colored milk. On all other measures the children's panel did not show significant differences between strawberry flavored and strawberry colored milk. Strawberry flavored milk that was stored for six weeks (and had lost its color) was rated lowest on all questions (even tying with strawberry colored milk for amount of strawberry flavor). To approach a definitive conclusion for this hypothesis, we need to look at the findings of hypotheses 1 and 2. The liking of strawberry milk by children had more to do with the decrease of sweet intensity and the increase in undesirable flavors than it had to do with the flavor itself. The decrease in the presence of color was confounded with time stored and the increase in undesirable flavors, therefore we cannot determine the causative factor.

Further Research

Further research can be conducted to separate the disappearance of color from the increase in undesirable flavor either by serving milk that is flavored, but not colored, or by coloring all milk just prior to tasting. Further research could also delve more into whether adding strawberry flavor just prior to tasting, rather than before pasteurization, is feasible from an industry standpoint.

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Appendix 1

Commands for R for statistical analysis of GC/MS data

```
R : Copyright 2006, The R Foundation for Statistical
Computing
Version 2.3.1 (2006-06-01)
ISBN 3-900051-07-0

R is free software and comes with ABSOLUTELY NO WARRANTY.
You are welcome to redistribute it under certain conditions.
Type 'license()' or 'licence()' for distribution details.

  Natural language support but running in an English locale

R is a collaborative project with many contributors.
Type 'contributors()' for more information and
'citation()' on how to cite R or R packages in publications.

Type 'demo()' for some demos, 'help()' for on-line help, or
'help.start()' for an HTML browser interface to help.
Type 'q()' to quit R.

> d<-read.csv("csv12del2.csv")
> d$Block<-as.factor(d$Block)
> d$Weeks<-as.factor(d$Weeks)
> m3<-aov(acetone~Block+Treatment*Weeks, data=d)
> summary(m3)
> coef(m3)
> TukeyHSD(m3, "Weeks")
> > m4<-aov(butanone~Block+Treatment*Weeks, data=d)
> summary(m4)
> coef (m4)
> TukeyHSD(m4, "Weeks")
> > m6<-aov(ethanol~Block+Treatment*Weeks, data=d)
> summary(m6)
> coef(m6)
> TukeyHSD(m6, "Weeks")
> > m8<-aov(ethyldimethylprop~Block+Treatment*Weeks, data=d)
> summary(m8)
> coef(m8)
> TukeyHSD(m8, "Weeks")
```

Commands are repeated for all compounds

Appendix 2

Commands for SAS for statistical analysis of GC/MS data

```
Imported file csv12del2dotlog.csv
```

(renamed `WORK.dna`)

```
proc contents;
run;
proc glm;
class block treatment weeks;
model acetone=block weeks treatment treatment*weeks;
lsmeans treatment weeks;
lsmeans treatment*weeks;
run;
```

Repeated substituting the following for acetone

```
butanone
ethylbutyrate
ethylmethylbutyrate
ethylhex
log_hydroxyprop_
methylmethylbutylbutyric
cishexenol
butyricacid
log_methylbutyricacid_
loghexacid_
ethylmethylphenylgly
gammadecalactone
```