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Juan He for the degree of Master of Science in Food Science and Technology
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Title: Flavor Comparison of Ultra High Temperature Processed Milk Heated by
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Abstract approved:

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Ultra high temperature (UHT) processing can extend shelf life of milk to several
months without refrigeration, which is more convenient and energy saving than
pasteurized milk. However, the poor acceptance caused by "cooked" flavors limits
its marketing growth, especially in United States. Ohmic heating, which has a
more uniform and rapid heating than conventional UHT process, may minimized
the flavor change during the thermal treatment. Flavor composition between
Ohmic heated UHT milk and other traditionally processed UHT milk (direct steam
injection and indirect plate heating) during 36 weeks storage were investigated in
this study. A total of 20 volatile compounds were analyzed based on their
importance to UHT milk as well as their representation to different chemical
classes including sulfur-containing compounds, ketones, lactones, aldehydes and

others. Dimethyl sulfide (DMS) and methyl ketones were significant different among three types of UHT heated milk. δ-lactones showed higher amount in Ohmic heating after stored for four weeks, which might generate creamy, fruity intermediate aroma. Other compounds showed no significant difference among three heating processes. Aroma recombination test revealed that the overall aroma of the ultra pasteurized (UP) milk could be mimicked by recombining 15 important reference odorants in the same concentrations as they occurred in the UHT milk using commercial pasteurized milk as the matrix.

Flavor Comparison of Ultra High Temperature Processed Milk Heated by Ohmic
Heating and Conventional Methods

by

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Juan He, Author

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CONTRIBUTION OF AUTHORS

This study was in collaboration with professors and students from Utah State

University. Dr. Donald J. McMahon designed and organized overall project. Dr

Silvana Martini and performed sensory analysis with trained panel and consumer

panels. Dr.Brothersen worked on milk processing.

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Chapter 1 INTRODUCTION

1.1 Flavors and off flavors in milk

Milk is composed of water (87.3%), milk fat (3.7%), lactose (4.6%), protein (3.25%), mineral substances (0.65%), organic acids (0.18%), and miscellaneous substances (0.14%). It provides significant amounts of protein and most micronutrients including calcium, vitamin Bs, vitamin A, iodine, magnesium, phosphorus, potassium, and zinc. The nutritional benefits of cow's milk make it an important source of nutrients in daily diets. It helps to improve the bone and dental health, and reduce the blood pressure, therefore prevent dental caries, osteoporosis, and hypertension. The calcium in milk products aids in reducing the risk of colon cancer (Wells, 2001).

Besides fulfilling nutritional requirements, consumers also enjoy the delicate flavor of milk and other milk-derived dairy products. The flavor quality of milk becomes a key parameter of product quality as acceptance is largely dependent on flavor (Drake et al., 2006). Fresh milk is a rather bland product: it has a pleasant, slightly sweet aroma and flavor, and a pleasant mouthfeel and aftertaste. Since fresh milk has a very delicate flavor, any off-balance of the flavor profile can emerge into "off-flavor" which can be easily detected by the consumer. The flavor

of milk is influenced by a variety of genetic and environmental factors involved in milk production, including the genetics of the cow, the physical and physiological condition of the cow, the type of feed consumed by the cow, the environment around the cow and the milking area, and biological, chemical and enzymatic changes in milk during production and distribution (Franklin, 1951). The flavor composition of milk is complex; at least 400 volatile compounds have been reported in milk, covering a wide range of chemical classes including lactones, acids, esters, ketones, aldehydes, alcohols, furans, carbonyls, pyrazines, sulfur compounds, and aliphatic and aromatic hydrocarbons (Moio et al., 1994). The off-balance of these volatile compounds in milk, as well as the generation of some new "off-flavor" compounds, can cause off-flavor in milk. According to The Committee on Flavor Nomenclature and Reference Standards of the American Dairy Science Association, off-flavors in milk can be categorized into heated, light-induced, lipolyzed, microbial, oxidized, transmitted, and miscellaneous, as shown in **Table 1.1** (Shipe et al., 1978).

1.1.1. Lipolyzed flavors

Lipolyzed flavors, one of the most common off-flavors in milk and dairy products, are produced by the enzymatic hydrolysis of milk fat triglycerides, resulting in the accumulation of free fatty acids (FFAs) as major degradation products, as well as di- and mono-glycerides and possible glycerol (**Figure 1.1**). At one time this type of

flavor defect was described as rancid, which caused considerable confusion because of its association with lipid oxidation. This was eventually resolved by referring to it as hydrolytic rancidity as distinct from oxidative rancidity, which more closely described "oxidized flavors." Heat resistant lipases from psychotropic bacteria, predominantly *Pseudomanas* species, have also been associated with lipolyzed flavors (Saxby, 1992).

The sensory detection thresholds of FFAs have been investigated in both water and oil by several authors (Brennand, 1989, Patton, 1964, Siek et al., 1969, Urbach et al., 1972), although the values can vary widely due to different methods used in the studies. The values showed that short-chain FFAs have higher threshold values in water than in oil, possibly due to their higher solubility in water, corresponding to a lower vapor pressure in aqueous media than in an oil, or lipid matrix. Milk is an emulsion or colloid of butterfat globules, which can be considered as a water-based fluid. Butanoic (C_4), hexanoic (C_6), octanoic (C_8) acids are often described as rancid, sweaty (body odor), goat-like, and generally unpleasant, while decanoic (C_{10}) and dodecanoic (C_{12}) acids, are not as desirable due to their soapy and waxy aromas. From butanoic acid ($C_{4:0}$) to octanoic acid ($C_{8:0}$), the sensory thresholds in a water phase generally increase with increasing chain length. The concentrations of butanoic (C_4), hexanoic (C_6), octanoic (C_8), decanoic (C_{10}), dodecanoic (C_{12}) acids are below their sensory thresholds (De Jong and Badings, 1990, Gonzalez-

Cordova and Vallejo-Cordoba, 2001) thus these compounds may not contribute to the overall aroma of fresh milk.

1.1.2 Microbial derived flavors

Milk is an ideal medium for microbial growth due to its various nutrients, so it is particularly important to utilize the most thorough sanitizing procedures as well as proper cooling and holding temperatures to ensure that the raw milk on the farm is of good quality. Off-flavors generally develop in processed milk when the bacterial population of the milk rises to $\geq 10^7$ CFU/ml (Schroder et al., 1982). Off-flavors development can classified into three stages: firstly, milk loses its freshness; secondly, it is perceived as stale; finally, its products generate rancid, fruity, and bitter flavors. Bitter flavors usually accompany protein degradation. Soapy and rancid flavors usually are a result of lipid breakdown (Cousin, 1982). Most microbial spoilage and associated off-flavors are due to post-pasteurization contamination generally involving psychrotrophic bacteria. Psychrotrophs are bacteria capable of growth at temperatures at or less than 7°C (44.6°F). Rapid cooling and refrigerated storage of raw milk has favored the growth of psychrotrophic bacteria in raw milk. During cold storage of milk they dominate the flora, and produce extracellular enzymes, mainly proteases and lipases, which is the major contribution to the spoilage of dairy products (Sørhaug and Stepaniak, 1997). For example, digestion of casein by proteases can generate a bitter flavor

and the gelation of milk. Lipases hydrolyze milk fat to produce free fatty acids, which cause milk to taste rancid, bitter, unclean, and soapy. Lecithinase degrades milk fat globule membranes and increases the susceptibility of milk fat to the action of lipases. A better understanding of bacterial-induced spoilage is needed for development of shelf-life prediction procedures.

Psychrotrophic bacteria from numerous genera have been isolated from milk, both Gram-negative (eg. *Pseudomonas*, *Aeromonas*, *Serratia*, *Acinetobacter*, *Alcaligenes*, *Achromobacter*, *Enterobacter*, *and Flavobacterium spp.*) and Gram-positive (eg. Bacillus (Meer et al., 1991), *Clostridium*, *Corynebacterium*, *Microbacterium*, *Micrococcus Streptococcus*, *Staphylococcus*, and *Lactobacillus* spp. (Champagne et al., 1994, Shah, 1994)). Of these, *Pseudomonas* is the most frequently reported psychrotroph in raw milk. The growth of the *Pseudomonas* strains and their concomitant production of proteases were reported as the cause of the release of plasmin and plasminogen from the casein micelle into the whey fraction (Fajardo-Lira and Nielsen, 1998, Nielsen, 2002). Eight unique milk spoilage aromas: rotten, barn, shrimpy, medicinal, fruity, cheesy, cooked, and overall spoilage aroma were used to differentiate milk spoiled by *Pseudomonas* strains. Ps. *fragi* was confirmed to produce fruity aromas in milk, while *Ps. putida* produced fruity, cheesy, rotten, and barn aromas. Spoilage aroma characteristics were found not only characterized by species, but influenced by species, fat level, and time (Hayes et al., 2002). It suggested that the extracellular enzyme activity

patterns among Pseudomonas isolates appeared to be associated with ribotypes

(Dogan and Boor, 2003). The potential early detection system of microbial

spoilage of milk-based products using an electronic nose unit including 14

conducting polymer sensors was studied with bacteria (*Pseudomonas*

aureofaciens, *P. fluorescens*, *Bacillus cereus*) or yeasts (*Candida pseudotropicalis*,

Kluyveromyces lactis) (Magan et al., 2001).

1.1.3 Proteolyzed flavors

Proteolytic enzymes degrade proteins and release a range of nitrogenous

compounds. Those proteases, which attack either casein or serum, will finally

result in the production of bitter flavors and the coagulation of milk. While

bacteria may be killed by heat treatment, certain heat-resistant enzymes

produced in raw milk by psychrotrophic bacteria may cause proteolytic and

lipolytic action. Proteolysis can be measured as the increase in trichloroacetic

acid-soluble free amino groups, and subsequently determined by trinitrobenzene

sulfonic acid (TNBS) via colorimetric analysis (Cogan, 1977). The relationship

between proteolysis measured with TNBS and off-flavor development was

investigated (McKellar, 1981), which showed TNBS method may be used as an

indicator of shelf life based on the fact that detection of proteolysis in milk prior to

development of off-flavors. Proteolysis can also be measured by the

determination of tyrosine value, which has been reported to associated with high

somatic cells counts (SCC) in milk (Senyk et al., 1985). The aromatic proteolytic-

derived products were reported to include p-cresol, methional, phenethanol,

phenylacetaldehyde, 3/2-methylbutanal, and 2-methylpropanal (Dunn and Lindsay,

1985). The different proteolytic-associated aroma could be affected by proteolytic

activity, either species differences (Stead, 1986) or environmental conditions

(Matselis and Roussis, 1998).

1.1.4 Oxidized flavors

Lipid oxidation influences the quality of food products through sensory

characteristics, such as flavor and taste deterioration, as well as reduction in

nutritive value. The flavors produced by oxidation of dairy products have been

described as being: oxidized, cardboard, beany, green, metallic, oily, fishy, bitter,

fruity, soapy, painty, rancid, grassy, buttery, tallow. "Oxidized flavor" was

recommended as the generic term to describe all these flavors (Shipe et al., 1978).

Lipid oxidation typically involves the reaction of molecular oxygen with

unsaturated fatty acids via a free radical mechanism or light-induced oxidation,

which influenced by factors such as degree of fatty acid unsaturation, content of

transition metal ions and antioxidants as tocopherols and carotenoids. The initial

products of lipid oxidation, lipid hydroperoxides, are quite bland but very unstable,

which can break down rapidly to produce short chain volatile flavor compounds,

such as hydrocarbons, acids, alcohols, aldehydes, and ketones which elicit undesirable flavors.

Polyunsaturated acids, including oleic, linoleic, linolenic and archidonic acid, are major precursors for the formation of aldehydes due to their prevalence in milk products. The possible origins of aldehydes produced by the autoxidation were listed in Table 1.2. In the autoxidation process, oxygen (O_2) is reacted with the methylene group adjacent to a double bond under catalysis of trace metals, such as copper, and by enzyme (Forss, 1979), leading to the formation of hydroperoxides, which further decompose to straight-chain aldehydes.

Light-induced off-flavor (LIOF) development is dependent on the availability of oxygen and ultraviolet light. LIOF are due to photosensitization of lipids and amino acid sulfur groups and are closely related to the wavelength and intensity of the light, exposure time, product temperature and the light transmission properties of the container. The major two distinctive off-flavors in milk induced by irradiation of light energy are sunlight and cardboard flavor. Sunlight flavor gives a burnt and oxidized odor in milk with light exposing for more than two days. Dimethyl disulfide (DMDS) and methional derived from oxidation of sulfur containing amino acids, such us methionine, are reported to be responsible for this flavor. Cardboard-like or metallic flavors, which develop in milk with prolonged duration of light exposure, are caused by secondary lipid oxidation products including

aldehydes, ketones, alcohols and hydrocarbons (Gaafar and Gaber, 1992).

Dynamic Headspace Analysis was used to evaluated major volatile compounds

from photosensitization and decomposition of riboflavin and oxidation of lipids

and sulfur compounds, included hexanal, pentanal, DMDS, 2-butanone, and 2-

propanol (Kim and Morr, 1996). Packaging can directly prevent the development

of light-induced off-flavor by protecting the product from both light and oxygen.

All plastic containers, such us polyethylene terephthalate (PET) package and high

density polyethylene (HDPE) package, can provide excellent convenience through

easy opening and reclosing, thus minimizing recontamination. Pigmented PET

bottles have excellent mechanical properties, a good barrier to oxygen and light

on milk quality. And pigmented HDPE bottles, both monolayer and multilayer, at a

higher thickness than current PET, are more suitable for the fresh milk packaging

market (Cladman et al., 1998). Chemical and sensorial aspects were studied in low

fat pasteurized milk bottled by various packaging materials (Moyssiadi et al., 2004).

Multilayer pigmented, monolayer pigmented, clear PET and pigmented PET were

compared with paperboard cartons for a period of 7 days. The result showed that

all packaging materials with regard to microbiological and chemical parameters

assessed good protection of milk over the test period. The multilayer provided the

best overall protection for the product, and followed by the monolayer TiO_2-

pigmented HDPE bottle. The degradation of volatiles by chemical reactions could

be one of the major reasons for the cause, such as oxidation caused by diffusion of

oxygen and light transmission into the packaging. Another reason is the interactions of aroma compounds with the packaging material itself. Lactones, aldehydes and free fatty acids, which were confirmed as important odor-active compounds in UHT-milk, had found weak tendency to adsorb to a glass bottle, whereas strong adsorption to a polyethylene packaging (Czerny and Schieberle, 2007). Comparative studies were conducted to access the sensory differences in milk packed with different materials (Boccacci Mariani et al., 2006). No off-flavor developed in milk packed in the paperboard was found during the storage period studied, however, a taint off-flavor was found developed in PET bottle (due to light-induced oxidative changes) after 1–2 days of storage (as assessed by the trained panel) and 2–3 days of storage (as assessed by consumers).

1.1.5 Processing caused flavor

1.1.5.1 Heat induced effect

Thermal treatment can destroy spoilage bacteria and inactivate enzymes then increase the shelf life of milk. The most popular thermal treatments, pasteurization and ultra high temperature (UHT), develop new volatile compounds which bring desirable, undesirable flavor, and also affect the texture and taste. UHT process is the sterilization of food by heating it for an extremely short period, around 1–2 seconds, at a temperature exceeding 135°C (275°F). While high temperature short time (HTST) pasteurization, which aims to destroy viable

pathogens so they are unlikely to cause disease instead of intending to kill all

microorganisms in the food, heats the food at lower temperature than UHT milk,

72°C (161.6°F) for at least 15 seconds. The main volatile compounds contributing

to heated flavor are Maillard reaction products. Thermal degradation of lipids

generate stale or oxidized flavor during storage. Thermal reactions involving

amino acid side chains generate sulfur compounds which are responsible for off-

flavor in UHT milk. Other thermal induced reactions such as hydrolysis of peptide

bonds, dephosphorylation of proteins, as well as the interaction of lipid oxidation

and Maillard reaction may also affect the flavor to some extent. Heat treatments,

particularly UHT process, can promote the development of thermally derived off-

flavor compounds such as aldehydes, methyl ketones and various sulfur

compounds (Contarini and Povolo, 2002, Contarini et al., 1997, Jeon et al., 1978,

Moio et al., 1994, Scanlan et al., 1968).

1.1.5.2 Non-thermal processing effect

New processing technology against thermal processing is needed to increase shelf

life of milk without compromising its natural flavor. Thermal processing is the

prevailing method to achieve microbial safety and shelf life stability of milk.

Although HTST pasteurization of milk is acceptable to most consumers, the

process does impart a slight cooked, sulfurous note, and the final product shelf life

is only 14-20 days at refrigeration temperatures. The promising non-thermal

method including membrane filtration, high pressure processing (HPP), pulsed electric field treatment are used to achieve a microbial shelf life similar to that of UHT milk and minimized the generation of off-flavor compounds. A similar microbiological reduction to that of pasteurized milk has been achieved using pressure treatments of 400 MPa for 15 min or 500 MPa for 3 min at room temperature (Rademacher and Kessler, 1996). At moderate temperature (55°C), HPP (586 MPa for 5 min) can significantly extend the shelf life of milk up to 45 days, which is beyond that of pasteurized milk (Tovar-Hernandez et al., 2005). Although it is generally assumed that HPP at low temperature will not change the aroma or flavor of the product, HPP under certain conditions has been reported to change concentration of some important flavor compounds. It was found that pressure, temperature, time, as well as their interactions all had significant effects ($P < 0.001$) on off-flavor generation including aldehydes, methyl ketones and sulfur compounds in milk (Vazquez-Landaverde et al., 2006b).

1.2 Ultra-high temperature processing milk

Ultra-high temperature processing (UHT) is the mostly wide used sterilization technique of food by heating it for a short period (normally hold for 1-2 seconds), at a temperature exceeding 135 ^{o}C (usually 138-150°C), which is the temperature required to kill spores in milk. After thermal treatment, milk is cooled quickly, and

then packed in sterile packaging under sterile conditions. The shelf life can last for

six to nine months without refrigeration (Chapman and Boor, 2001).

The growth of UHT milk consumption has been noticeable in the past 20 years

especially in Europe, Asia, and South America. UHT milk gained popularity in these

countries as an alternative to pasteurized milk due to environmental factors or

limited availability of milk supplies and refrigeration. The most significant benefit

in consuming UHT milk is convenience. The high thermal treatment and aseptic

package yield the product to last for several months without refrigeration until

open. However, this shelf-stable milk has met with limited acceptability by the

consumer in the United States. The flavor change in the UHT milk, the persistency

to pasteurized milk, and the higher cost of UHT milk (Pearson et al., 1990) may be

the reasons why the acceptance of UHT milk has been slow in US.

Otherwise, Americans distrust milk that has not been refrigerated. Milk under cold

temperature is preferred more than room temperature UHT milk to drink. When

the largest UHT milk manufacturer in the world, Parmalat, first introduced UHT

milk to the U.S. market back in 1993, poor feedback was received. Customers

distrusted the milk stored without refrigeration and refused to drink milk at room

temperature. In order to overcome the dislike from the unfamiliarity, the milk was

sold in normal packaging and in the refrigerator aisle, and labeled as "ultra

pasteurized" milk. Now, almost all of the organic milk and the majority of conventional milk available in U.S. supermarkets is UHT processed.

1.2.1 Conventional heating methods

Direct steam injection heating is applied by injecting the discharge of steam bubbles into a liquid at a lower temperature to transfer heat. It's characterized by high heating and cooling rates and short holding time. It has been used as an effective heating method to improved shelf-life and increased freshness, sweetness and general acceptance in comparison with pasteurized milk (Fredsted and Rysstad, 1995). The subsequent evaporative cooling procedure, applied in direct heat treatment technologies to remove the excess water added as steam during heating, has an additional deodorizing effect, which may reduce the content of some volatile compounds in the products (Contarini et al., 1997).

Conventionally, the heating process can also be done by using steam, hot water or thermal oil to flow through the plate heat exchanger, termed "indirect plate heating" which provides good mixing and high convective heat transfer coefficient, to heat the material by conduction.

1.2.2 Ohmic heating processing

Ohmic heating is the thermal process that uses the passage of an alternating electric current through the food material, which serves as an electrical resistor, to

release heat. The instrumental design usually consists of electrodes that contact the food material, whereby electricity is directly passed through the substance using a variety of voltage and current combinations. Electrical energy is dissipated into heat without the use of hot heat transfer surfaces, which results in rapid and uniform heating. Ohmic heating is also termed "electrical resistance heating", "Joule heating", or "electro-heating", and may be used for a variety of applications in the food industry.

1.2.2.1 The history of Ohmic heating applied to milk

The principle of this process can be traced back to 19th century, when James Prescott Joule studied Joule's First Law in 1841. It's a physical law expressing the relationship between heat generated by the current flowing through a conductor of electrical resistance. Joule heating is referred to as Ohmic heating or electrical resistive heating because of its relationship to Ohm's Law. And several processes were patented that used electrical current for heating liquids.

In the late 1920's, pasteurization of milk was introduced to the United States. It was achieved by electricity when passing milk between voltaged parallel plates (Anderson and Finkelstein, 1919). Six states in the United States had approximately fifty commercial electrical sterilizers in operation at that time (Sastry and Palaniappan, 1992b). Unfortunately, this technology virtually disappeared in 1950's due to lack of the suitable inert electrode material and

improper contact between electrodes and the food product (De Alwis and Fryer, 1990). Based on new and improved materials and designs for Ohmic heating, most recent achievement for this technology was the continuous-flow Ohmic heater and licensed the technology to APV Baker patented by the Electricity Council of Great Britain (Skudder, 1988). Then a considerable amount of attention has been given for Ohmic heating in aseptic processing of liquid-particulate foods.

1.2.2.2 The principle of Ohmic heating

Ohmic heating can be used for heating food that is an electrically conducting moist material. For example, milk, which contains water and ionic salts, is capable of conducting electricity, but it also has a resistance which generates heat when an electric current is passed through it. Therefore, the rate of heating is directly proportional to the square of the electric field strength and the electrical conductivity. The electric field strength is controlled by adjusting the electrode gap or the applied voltage. However, the heating rate depends largely on the physical characteristics of the food itself, especially on the electrical conductivity (De Alwis and Fryer, 1992).

Like conventional heating, microbial inactivation in relation to Ohmic heating is primarily by heat.

1.2.2.3 Advantages of Ohmic Heating over conventional heating

Ohmic heating has numerous advantages over conventional heating methods. The conventional heating processes applied in this study were direct steam injection heating and indirect plate heating. In direct steam injection heating, the discharge of steam bubbles is injected into a liquid at a lower temperature to transfer heat. And in indirect plate heating, steam, hot water or thermal oil is used to flow through the plates to heat the material by conduction. While Ohmic heating heats the entire mass of the food material volumetrically because of its inherent electrical resistance (Sastry and Palaniappan, 1992a). The differences between the principles of these heating procedures decide the heating effects. The way that how conventional heating methods work make them struggle to achieve the necessary high rates of heat transfer into the material in order to heat sufficiently without causing product degradation. Therefore, the most important advantage of Ohmic heating is the rapid and uniform heating. With Ohmic heating, the resulting product is heated rapidly (increase $50^{o}C$ in less than 0.1 second) and without temperature gradients.

Another particular advantage over conventional heating are problems such as fouling, "burn-on" where thermal degradation occurs as a result of high temperature gradients. During conventional heating methods, significant product quality damage may occur due to slow conduction and convection heat transfer.

Furthermore, the time takes to increase the temperature at the coldest point which is generally the center of the largest particle, may over-process the remaining particles and the surrounding liquid. But during Ohmic heating, the voltage being applied can be easily and rapidly changed, the product temperature can be very accurately controlled and no residual heating occurs when the current is shut off. And hot surfaces for heat transfer are eliminated, resulting in optimized time and temperature control profiles with less overcooking, thus fouling and "burn-on" of such surfaces are greatly reduced, offering less maintenance and better product flavor (Parrott, 1992).

Additionally, it is possible to process large particulate foods (up to 1 inch) with Ohmic heating that has a larger heat penetration depth compared to other heating techniques (Fryer et al., 1993), like microwave and radio frequency heating.

Ohmic heating is also favored for higher energy conversion efficiency, because up to 90% of the electrical energy is converted into heat and lower in capital cost due to reduced moving parts. Ohmic heater cleaning requirements are comparatively less than those of traditional heat. Fouling of the system or scorching of the product is minimized and Ohmic heating systems have the potential for operating for longer time than indirect heat exchangers in which fouling and build up of a bio-film requires frequent cleaning to be performed.

Table 1.1 Categories of off-flavors in milk

Cause	Descriptive or associated terms
Heated	Cooked, caramelized, scorched
Light-induced	Light, sunlight, activated
Lipolyzed	Rancid, butyric, bitter, goaty
Microbial	Acid, bitter, fruity, malty, putrid, unclean
Oxidized	Papery, cardboard, metallic, oily, fishy
Transmitted	Feed, weed, cowy, barny
Miscellaneous	Flat, chemical, foreign, lacks freshness, salty

Table 1.2 Possible origins of aldehydes obtained from specific unsaturated fatty acids

Unsaturated fatty acids	Aldehyde obtained
Oleic acid	Octanal, nonanal, decanal, 2-decenal, 2-undecenal
Linoleic acid	Hexanal, 2-octenal, 3-nonenal, 2,4-decadienal
Linolenic acid	Propanal, 3-hexenal, 2,4-heptadienal, 3,6-nonadienal, 2,4,7-decatrienal
Archidonic acid	Hexanal, 2-octenal, 3-nonenal, 2,4- decadienal, 2,5-undecadienal, 2,5,8-tridecatrienal
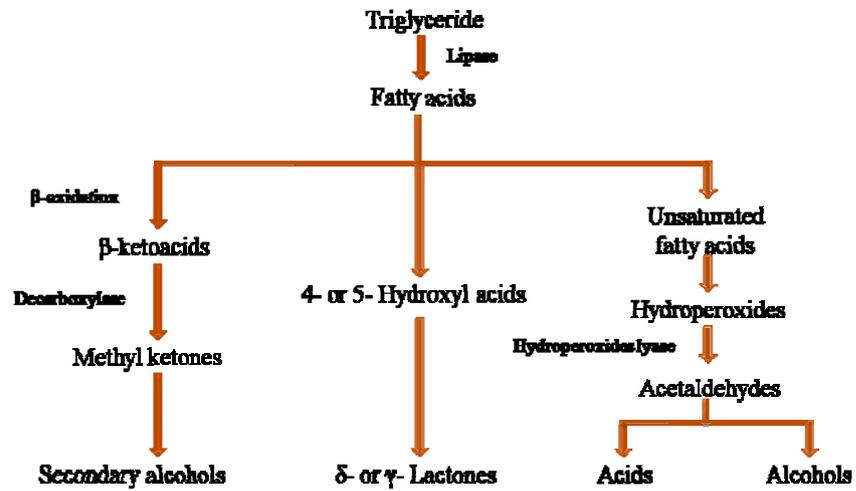
Figure 1.1 General pathways for the metabolism of milk triglycerides and fatty acids.

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Chapter 2 QUANTITATIVE MEASURMENTS ON VOLATILE AROMA COMPOUNDS IN UHT MILK TREATED BY OHMIC HEATING AND CONVENTIONAL METHODS

2.1 Abstract

Flavor differences of Ohmic heating UHT milk with other traditionally processed UHT milk (direct steam injection and indirect plate heating) and their changes during storage were investigated in this study. A total of 20 volatile compounds classified in volatile sulfur compounds, ketones, lactones, aldehydes were monitored during 36 weeks. DMS and methyl ketones had significant difference on three types of UHT heated milk, which were highly related to the severity of heat treatment effect. δ-lactones showed higher amount in Ohmic heating after storing for four weeks, which might generate creamy, fruity intermediate aroma. Significant changes of aldehydes occurred in indirect plate heating milk after stored for 36 weeks, which may deepen the dislike of this type of UHT milk. Other compounds showed no significant difference among three heating processes.

Key words: Ohmic heating, direct steam injection, indirect plate heating, flavor, UHT milk.

2.2 Introduction

Thermal treatment can destroy spoilage bacteria and deactivate enzymes, therefore increase the shelf life of milk. The most popular thermal treatments, pasteurization and ultra high temperature (UHT) process, develop new volatile compounds which bring desirable or undesirable flavor, and affect taste and texture. Pasteurized milk typically has a shelf life normally last about 14 to 20 days at refrigeration temperatures, while ultra-high temperature (UHT) process allows milk to be stored at room temperature as long as 6 to 9 months (Burton, 1994). However, the higher temperature used in UHT process may generate undesirable flavors to many U.S. consumers (Blake et al., 1995). The main volatile compounds that contribute heated flavor are Maillard reaction products. Thermal degradation of lipids generates stale or oxidized flavor during storage. Thermal reactions involving amino acid side chains generate sulfur compounds, which are responsible for off-flavor in UHT milk. Other thermal-induced reactions such as hydrolysis of peptide bonds, dephosphorylation of proteins, as well as the interaction of lipid oxidation and Maillard reaction, may also affect the flavor to some extent. The development of thermally derived off-flavor compounds promoted by conventional UHT process such as aldehydes, methyl ketones and various sulfur compounds has been studied in the past decades (Contarini and Povolo, 2002, Contarini et al., 1997, Jeon et al., 1978, Moid et al., 1994, Scanlan et al., 1968).

Ohmic heating, also termed "electrical resistive heating" or "Joule heating", is an in-situ electrical heating technology that applies electric current through electrodes into a food product, which impedes current and causes it to give off heat. There are several advantages associated with Ohmic heating (Biss et al., 1989, Skudder and Biss, 1987). It provides very fast heating rates and the heating occurs throughout the entire food product through which the electrical current passes, heating occurs volumetrically and the product does not undergo large temperature variations or come in contact with surfaces hotter than the fluid itself (Sastry and Palaniappan, 1992a). Also because the voltage being applied can be easily and rapidly changed, the product temperature can be very accurately controlled and no residual heating occurs when the current is shut off. Thus, fouling of the system or scorching of the product is minimized and resistive heating systems have the potential for operating for longer time than indirect heat exchangers in which fouling and build up of a bio-film requires frequent cleaning to be performed.

Due to rapid and uniform treatment along with its high energy efficiency and technical simplicity, Ohmic heating has been applied to thermal processing of a variety of foods (De Alwis and Fryer, 1990, Palaniappan et al., 1990, Skudder, 1989). The temperature of liquid at the outlet of a resistive heating system is a controlled by the flow rate, electrical conductivity of the fluid, applied voltage gradient and dimensions of the heating unit (Qihua et al., 1993). Compared to the

conventional UHT process, the rapid heating obtained by Ohmic heating produces

fewer heat-induced changes than indirect heating, and does not have the flavor

evaporative cooling of direct steam injection. Very rapid and uniform heating is

possible to minimize heat-induced flavor changes by Ohmic heating. The aim of

this work was to compare the flavor differences of Ohmic heating UHT milk with

direct steam injection and indirect plate heating UHT milk and their changes

during storage, in order to test the hypothesis of improving UHT milk flavor by

Ohmic heating.

2.3 Materials and methods

2.3.1 Experimental Design

2% reduced fat ultra high temperature processed milk from the same batch using

three processing treatments (direct steam injection and indirect plate heating

processes were manufactured at Carine Dairy Research & Teaching Center Utah

State University; Ohmic heating was produced at Patterson in California and

delivered by tanker to the Gary H. Richardson Dairy Products Laboratory.

Triplicate trials were performed (replicate 1 produced by March 31, 2009,

replicate 2 by May 31, 2009 and replicate 3 by September 28, 2009) and stored at

room temperature 22$^{\text{o}}$C at Utah State University. Any samples that had lost

sterility during packaging and coagulated during storage were deleted from the study and sent for disposal. At 1, 4, 16 and 36 wk of storage, samples of milk were received at Oregon State University for flavor chemistry analysis. Volatile sulfur analysis was applied soon after the samples were received. After that milk samples were frozen at -10°F until all the samples were collected to perform volatile aroma analysis on aldehydes, ketones, and lactones at same time. Samples were thawed at room temperature before analyzing.

2.3.2 Chemicals

Sodium chloride, methanethiol (MeSH), dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), and isopropyl disulfide (IsoProDS), 2-methylpropanal, hexanal, heptanal, octanal, nonanal, 2-heptanone, 2-nonanone, 2-undecanone, γ-nonalactone, γ-decalactone, γ-dodecalactone, δ-decalactone, β-ionone were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethyl methyl sulfide (EMS), dimethyl sulfide (DMS), δ–undecalactone, δ–dodecalactone were supplied by TCI America (Portland, OR, USA). Benzaldehyde was bought from Polyscience Corp (Niles, IL). Methanol was obtained from J.T. Baker (Phillipsburg, NJ, USA).

2.3.3 Flavor analysis of volatile compounds

2.3.3.1 Analysis of volatile sulfur compounds by headspace-SPME-GC-PFPD

Dimethyl sulfide, methanethioil, dimethyl disulfide and dimethyl trisulfide were analyzed using headspace solid-phase microextraction and gas chromatography with pulsed-flame photometric detection (HS-SPME/GC-PFPD) technique previously developed (Vazquez-Landaverde et al., 2006a). An aliquot of 2 ml of milk sample was diluted with 8 ml water which was saturated with sodium chloride, and then added to a 20 ml deactivated vial. 50 μL internal standard (100 μg/kg EMS and 190 μg/kg isopropyl disulfide) was added. Triplicate analyses were performed for each sample. Volatile sulfur compounds in milk were extracted with a 1 cm 85 μm carboxen/ polydimethylsiloxane SPME fiber (Supelco Co., Bellefonte, PA). A CombiPAL SPME autosampler (CTC Analytics AG, Zwingen, Switzerland) and a CP-3800 gas chromatograph (Varian, Walnut Creek, CA) equipped with a pulsed flame photometric detector (PFPD) were used. The sample was pre-equilibrated at 35 ^{o}C for 5 min with agitation at 500 rpm, and the volatile sulfur compounds were extracted at the same temperature for 20 min with agitation 250 rpm. The volatile compounds extracted by the SPME fiber were thermally desorbed at 300 ^{o}C in the GC injector in splitless mode (1:5 split ratio was chosen for DMS quantification due to its higher concentration). Separation of the analytes was achieved using a DB-FFAP fused silica capillary column (30 m x 0.32 mm, 1.0 μm

film thickness, Agilent, Palo Alto, CA) with a constant nitrogen flow of 2 ml/min.

The oven temperature program was as follows: 35 ^{o}C held for 5 min, heated to

150 ^{o}C at a rate of 10 ^{o}C/min, held for 1 min, and then heated to 220 ^{o}C at a rate

of 20 ^{o}C /min with a final hold time of 5 min. The PFPD was held at 300 ^{o}C and 500

V with the hydrogen flow rate at 14 ml/min, air 1 flow rate at 17 ml/min, and air 2

flow rate at 10 ml/min. The detector response signals were collected and

integrated using computer software (Star Workstation 6.2, Varian).

Chromatographic identification of target sulfur compounds was performed by

comparing retention times to those of pure standards. Standard calibration curves

were obtained by standard addition technique and the correlation coefficients (R^2)

of each target compound were above 0.990 (Table 2.1). The stock solutions were

prepared by dissolving around 10^4 mg/L each target compound individually into

methanol solution. Individual stock solution was added to methanol to make the

first level mixed standard solution, which was then diluted at 1:1 (v/v), 3:5 (v/v),

1:3 (v/ v), 1:7 (v/ v), 1:15 (v/ v) and 1:31 (v/ v) ratio with methanol to give a range

of concentrations. 50μL standard solutions of each level were added and analyzed

using the same procedure as described for the milk sample. Square root ratios of

the standard to its corresponding internal standard were plotted. Concentrations

were calculated based on the squared root of the peak area ratio of the

compound to the internal standard.

2.3.3.2 Analysis of aldehydes by headspace-SPME-GC-MS

2-methylpropanal, hexanal, heptanal, octanal, nonanal and benzaldehyde were analyzed using headspace solid-phase microextraction and gas chromatography with mass spectrometry detection (HS-SPME/GC-MS) technique. 10 ml of sample with 20 µL internal standard (96 mg/L 3-heptanone) was extracted in a 20 ml deactivated vial with a 2 cm 50/30 µm divinylbenzene/ carboxen/ polydimethylsiloxane SPME fiber (Supelco Co., Bellefonte, PA). The analysis of volatile compounds was carried out by using an Agilent 6890 gas chromatograph equipped with a 5973 mass selective detector (Agilent Techonologies, Inc., Wilmington, DE) and a Gerstel MPS-2 multipurpose autosampler (Gerstel USA, Baltimore, MD). The sample was pre-equilibrated at 50 ^{o}C for 5 min with agitation at 250 rpm, and the volatile compounds were extracted at the same temperature for 60 min with agitation 250 rpm. The volatile compounds extracted by the SPME fiber were thermally desorbed at 300 ^{o}C in the GC injector in splitless mode. Separation of the analytes was achieved using a DB-5 fused silica capillary column (30 m × 0.25 mm, 0.5 µm film thickness, Agilent, Palo Alto, CA) with a constant nitrogen flow of 1.1 ml/min. The oven temperature program was as follows: 40 ^{o}C held for 2 min, heated to 120 ^{o}C at a rate of 2 ^{o}C/ min, and then increased to 250 ^{o}C at a rate of 26 ^{o}C/ min, held for 5 min. Standard EI mode was used at 70 eV. The total mass ion chromatogram was obtained from 40 to 350 amu. System software

control and data management/analysis were performed through Enhanced ChemStation Software (Agilent Techonologies, Inc.). Compounds were identified through mass spectra. Selective ion-monitoring (SIM) mass spectrometry was used to quantify the aroma-active compounds. Standard calibration curves were obtained by standard addition technique. The stock solutions were prepared by dissolving around 104 mg/L each target compound individually into methanol solution. Individual stock solution was added to methanol to make the first level mixed standard solution, which was then diluted at 1:1 (v/v), 3:5 (v/v), 1:3 (v/ v), 1:7 (v/ v), 1:15 (v/ v) and 1:31 (v/ v) ratio with methanol to give a range of concentrations. 20μL standard solutions of each level were added and analyzed using the same procedure as described for the milk sample. The quantifying and qualifying ions for both the aroma-active compounds and internal standards were listed in Table 2.2. The calibration curve for individual target compounds was built up by plotting the selected mass ion abundance ratio of target compounds with their respective internal standard against the concentration ratio. For each calibration curve, the regression correlation coefficients (R^2) were calculated using the ChemStation data analysis software.

2.3.3.3 Analysis of methyl ketones and lactones by SBSE-GC-MS

2-Heptanone, 2-nonanone, 2-undecanone, β-ionone, γ-nonalactone, γ-decalactone, γ-dodecalactone, δ-decalactone, δ-undecalactone and δ-

dodecalactone were analyzed using stir bar sorptive extraction and gas

chromatography with mass spectrometry detection (SBSE/GC-MS) technique. A

stir bar (1cm long, 0.32mm OD and 0.5mm film thickness) with a

polydimethylsiloxane (PDMS) phase was used for the extraction of volatile

compounds. The stir bar was cleaned with 1:1 (v/v) methanol and dichromethane

by stirring for 30 min at 1000 rpm, allowed to air dry for 1 h, and then conditioned

for 30 min at 280 ^{o}C with 50 ml/min nitrogen flow. 20 ml of milk sample and 20 µL

internal standard (mixture of 29.39 mg/L 3-heptanone and 32.07 mg/L ethyl

cinnamate) were added to a 20 ml clear glass vials (I-Chem, New Castle, DE) with

polytetrafluorethylene septum caps. The milk sample was extracted at room

temperature for 3 h at 1000 rpm. The analysis of volatile compounds was carried

out by using an Agilent 6890 gas chromatograph equipped with a 5973 mass

selective detector (Agilent Techonologies, Inc., Wilmington, DE) and a Gerstel

MPS-2 multipurpose TDU autosampler with a CIS-4 cooled injection system

(Gerstel USA, Baltimore, MD). The analytes were thermally desorbed at the TDU in

splitless mode, ramping from 25 to 250 ^{o}C at a rate of 150 ^{o}C/min, and held at the

final temperature for 3 min. The CIS-4 was cooled to -80 ^{o}C with liquid nitrogen

during the sample injection, then heated at 10 ^{o}C/s to 250 ^{o}C for 10 min. Solvent

vent mode was used during the injection with a split vent purge flow of 50 ml/min

beginning at 3 min. The helium column flow was 2 ml/min. Separation was

achieved using a HP-5 column (60 m × 0.32 mm ID and 0.25µm film thickness,

Phenomenex, Torrance, CA). The oven temperature was programmed at 40 ^{o}C for

2 min, then ramped to 250 ^{o}C at a rate of 6 ^{o}C /min and held at the final

temperature for 10 min. Standard EI mode was used at 70 eV. The total mass ion

chromatogram was obtained from 35 to 350 amu. System software control and

data management/analysis were performed through Enhanced ChemStation

Software (Agilent Techonologies, Inc.). Compounds were identified through mass

spectra. Selective ion-monitoring (SIM) mass spectrometry was used to quantify

the aroma-active compounds. Standard calibration curves were obtained by

standard addition technique. The stock solutions were prepared by dissolving

around 104 mg/L each target compound individually into methanol solution.

Individual stock solution was added to methanol to make the first level mixed

standard solution, which was then diluted at 4:1(v/v), 1:1 (v/v), 2:3 (v/v), 1:3 (v/ v),

1:7 (v/ v), 1:15 (v/ v) and 1:31 (v/ v) ratio with methanol to give a range of

concentrations. 20μL standard solutions of each level were added and analyzed

using the same procedure as described for the milk sample. Selective ion-

monitoring (SIM) mass spectrometry was used to quantify the aroma-active

compounds. The quantifying and qualifying ions for both the aroma-active

compounds and internal standards are listed in Table 2.3. The calibration curve for

individual target compounds was built up by plotting the selected mass ion

abundance ratio of target compounds with their respective internal standard

against the concentration ratio. For each calibration curve, the regression

correlation coefficients were calculated using the ChemStation data analysis software.

2.3.4 Statistical Data Analysis

All data were recorded and summarized with Miscrosoft Excel 2007 software. Analysis of variance (ANOVA), Tukey HSD tests were carried out with R software (Version 9.2).

2.4 Results and Discussions

A total of 20 volatile compounds were selected based on their importance to UHT milk as well as their representation to different chemical classes including sulfur-containing compounds, ketones, lactones, aldehydes and others. The volatile composition of the UHT treated with different heating methods during 36 weeks storage time was listed in **Table 2.4** and **Table 2.5**. And the calculated Odor Activity values (OAV) were summarized in **Table 2.6**.

2.4.1 Sulfur compounds

The concentrations of sulfur compounds in milk are related to the severity of heat treatment of the milk. Thermal processing can generate cooked, sulfurous, cabbage-like off-flavors in milk. Researchers have identified that hydrogen sulfide

(H_2S), MeSH, carbon disulfide (CS_2), DMS, DMDS, DMTS were related with this cooked off-flavor defect (Datta et al., 2002, Shipe, 1980, Simon and Hansen, 2001). H_2S was not found in our study, which might be evaporated during the heating process due to low boiling point or might disappear after the first week storing. It was reported to have a rapid decreasing within the first week (Jaddou et al., 1978). And the first time point in this study started from the end of first week. CS_2 was not quantified due to its reported low concentration and minor aroma contribution in milk (Vazquez-Landaverde et al., 2006a).

Volatile sulfur compounds, like DMS, are present naturally in raw milk (Patton et al., 1956, Toso et al., 2002). They can also be formed from the sulfhydryl (-SH-) group of milk proteins subjected to thermal denaturation (Datta et al., 2002, Hoffmann and van Mil, 1997). Heat treatment causes milk fat globule membrane proteins and whey proteins to unfold the sulfhydryl groups, normally buried in the native protein, are exposed to the outer surfaces. The main source of volatile sulfur compounds was claimed to associate with β-lactoglobumin (β-Lg) decomposition, a major protein component of the whey fraction (accounting for over 50% of the total protein) (Hutton and Patton, 1952). It is highly thermal sensitive and will denature under heat treatment. The degree of denaturation is correlated with the severity of the heat treatment.

Among three UHT milk, indirect plate heating processed milk showed significantly

highest concentration of DMS, while steam heating processed milk had lowest

amount (P<0.05) (**Table 2.4**, **Table 2.5** and **Figure 2.1**). The observation indicated

that DMS was highly related to the severity of the heat treatment. This appeared

to reflect a difference in the heat treatment and the results were in consistent

with hypothesis. Plate heat exchangers applied in indirect plate heating associated

with low liquid velocities could lead to uneven heating and "burn-on", and then

would have higher heating temperature especially at the contact surface when

compared with other two treatments. Therefore, generated more heat related

compounds than Ohmic heating and direct steam injection, like DMS in this study.

After steam injection, the required followed procedure, evaporative cooling, could

cause the loss of volatile compounds. So that direct steam injection heating milk

had a slightly lower amount of volatile compounds than Ohmic heating milk. Much

higher DMS concentration in plate heating milk may generate undesirable rancid

flavor more than Ohmic and steam heating milk. And Ohmic heating milk was

higher than pasteurized milk, while steam heating milk was lower after stored for

one week (**Table 2.4**). Then Ohmic heating milk began to decrease when stored

longer, while steam heating milk increased. However DMS in milk was not favored

for low concentration, neither too much nor too little was preferred. The control

level in pasteurized milk might be a good indicator to show the favored

concentration. The closer to the control level, the better the milk was preferred.

And this hypothesis was proved by a separated sensory evaluation study in this project. The sample analyzed each time point in this study was subjected to the sensory preference evaluation. The results showed that UHT milk had similar preference with different heating processes at 1 week. UHT milk made using Ohmic heating was similar to direct steam injection milk with a slight preference at 4 and 16 week. At 36 week, indirect plate heating milk had least preference associated with highest oxidation score.

According to the magnitude of OAV (**Table 2.6**), MeSH, DMDS and DMTS could be the most important sulfur-containing contributor to the aroma of UHT milk. The concentrations were more than 50 times higher than their reported threshold in water. Since the milk constituent is way different than just water, especially in the absence of fat. These volatile compounds would dissolve more in milk compared to water, which caused fewer amounts spread into the headspace, therefore responsible for higher threshold in milk. The thresholds of DMS and DMDS in milk were reported as 20 and 21 μg/kg respectively, which were ten to hundred times higher in water. Then the calculated OAV would be ten to hundred times lower when thresholds were reported in milk, the real aroma contribution of these volatile sulfur compounds may not play that extraordinary important role in milk.

MeSH has a strong and unpleasant cabbage, sulfur-like aroma. It is thought to be liberated during heat treatment from methionine, by breakdown of the sulfur

bearing side chain but the actual pathway has not been well elucidated. The presence of DMDS and DMTS were claimed to be formed most from MeSH oxidation. In this study, MeSH did not show significant difference among these three types of UHT milk (**Table 2.4** and **Table 2.5**). Both DMDS and DMTS had very low concentration (~1μg/L) and did not affected by the different ways of heating, and did not accumulate during storage. Significant variations were obtained for DMDS and DMTS, which might caused by the oxidation happened in milk sample or the variation among milk replications (**Table 2.4** and **Table 2.5**)..

2.4.2 Methyl ketones

2-heptanone, 2-nonanone and 2-undecanone were quantified by SBSE-GC-MS method mentioned above, which were reported the largest contributors of stale flavor in UHT milk (Badings and Neeter, 1980, Badings et al., 1981). Methyl ketones are primarily formed by thermal oxidation of milk fat. They are formed during heat treatment from the oxidation of free fatty acids into ß-ketoacids, and the subsequent decarboxylation into methyl ketones (Moio et al., 1993). 2-heptanone and 2-nonanone had been identified as the most abundant volatile flavor compounds in UHT milk (Moio et al., 1994). The effect of heat treatments had been studied on volatile compounds in commercially processed milk samples (Contarini and Povolo, 2002). Methyl ketones exhibited a correlation with the

severity of the heat treatment. In another study, their concentrations were measured markedly higher in UHT milk than in raw and pasteurized milk (Vazquez-Landaverde et al., 2005). And these compounds could have very important contributors to the aroma of heated milk based on their high odor activity value (OAV) in UHT milk (from 4-10).

In this study, UHT milk had higher amount of methyl ketones than pasteurized milk. Indirect plate heating milk showed significantly higher concentrations ($P<0.05$) in all these three methyl ketones than direct steam injection and Ohmic heating milk (**Table 2.4**, **Table 2.5, and Figure 2.2**), which had a very similar pattern of DMS mentioned above. And direct steam injection generated lowest concentration among these three UHT processes. It indicated that methyl ketones were highly correlated with the heat severity of heating process.

And all these compounds showed an increasing trend when stored. The increase in total methyl ketones was greater in steam (153.3±26.6%) and Ohmic heating milk (100.1±13.0%), while less in plate heating milk (23.2±4.1%).

2.4.3 Lactones

Lactones are originally found in very small amounts in unheated milk, and are formed in the ruminant mammary gland from the hydrolysis of saturated fatty

acids and subsequent cyclisation of free hydroxyacids (Dumont and Adda, 1979).

They are cyclic esters that formed during heat treatments from the thermal

breakdown of γ- and δ-hydroxyacids through intramolecular esterification of

hydroxyacids where the loss of water results in ring formation (Fox et al., 2000).

Thus could be important to the flavor of heated milk. Especially for the strong

creamy aroma that associated with cooked flavor. Six lactones (γ-nonalactone, γ-

decalactone, γ-dodecalactone, δ-decalactone, δ-undecalactone and δ-

dodecalactone) were identified in this study. Based on the structure difference, δ-

lactones (**Figure 2.3a**) had different generation trend from γ -lactones (**Figure**

2.3b). However, all three γ- lactones quantified in this study, γ-nonalactone, γ-

decalactone, γ-dodecalactone expressed very similar developing pattern, so as all

three δ - lactones (**Table 2.4** and **Table 2.5**). γ -lactones decreased during storage.

Pasteurized milk generated lower concentrations of both δ -lactones and γ-

lactones than UHT processes. δ -lactones had highest concentration at 4 weeks,

then rapidly decreased. The developing pattern followed intermediate flavor -

"heated" flavor, which developed over 1-3 weeks and was considered as optimum

flavor of UHT milk. It's worth noting that Ohmic heating milk developed highest

amount in γ-, δ-dodecalactone ($p<0.5$) only in 4 weeks. The phenomenon again

gained support from sensory evaluation study that Ohmic heating received

highest preference since 4 weeks. Besides of which, there was no significant

difference among these three types of milk at each storage time overall.

2.4.4 Aldehydes

Alkanal compounds and Strecker aldehydes being the largest contributors to
casein derived stale flavors were considered particularly problematic. Four alkanal
compounds (hexanal, heptanal, octanal, nonanal) and two Strecker aldehydes (2-
methylpropanal, benzaldehyde) were quantified in this study. Aldehydes appeared
to have less effect by heat processing than ketones. The formation of alkanal
compounds during storage resulted from the auto-oxidation of unsaturated fatty
acids ($C_{18:1}$ and $C_{18:2}$) and also the spontaneous decomposition of hydroperoxides
promoted by heat (Grosch, 1982). Hexanal and heptanal were the only two
aldehydes detected in pasteurized milk in this study, which had lower
concentration than in UHT processed milk (**Table 2.4**). No clear pattern of
formation for these various aldehydes in UHT milks when processed by plates,
steam or electrical resistance methods (**Table 2.4** and **Table 2.5**). Hexanal, octanal
and nonanal showed overall decreasing trend, while heptanal increased during
storage. Elevated levels of Strecker aldehydes can be produced due to the
Strecker degradation of amino acids during Maillard reactions (Damodaran, 1996).
They increased during storage and had lowest concentration in UHT milk by Ohmic
heating. Changes in most aldehydes were significant for indirect plate heating milk,
which showed highest concentrations at 36 week, and may deepen the dislike of
plate heating milk. Stale flavor perception may be related to the change in

threshold levels for aldehydes as lactone levels decreased during storage (Adhikari

et al., 2006).

Table 2.1 Regression equations for volatile sulfur compounds in milk

Compounds	Internal standard	Regression equation[1]	R^2
DMS	EMS	y = 0.426x	0.998
DMDS	IsoProDS	y = 3.247x	0.996
DMTS	IsoProDS	y = 2.959x	0.996

[1]y = square root of response of target compound/ square root of response of internal standard;

x = [compound]/[internal standard].

Table 2.2 Regression equations for milk flavor compounds extracted by HS-SPME-GC-MS method

Compounds	Quantify ion	Qualify ion	Regression equation[1]	R^2
2-methylpropanal	43	57, 72	y=0.0413x	0.995
benzaldehyde	106	77	y=0.260x	0.996
hexanal	56	72, 82	y=0.260x	0.999
heptanal	70	55	y=0.274x	0.997
octanal	56	69, 84	y=0.411x	0.993
nonanal	57	70, 98	y=0.220x	0.991

[1]y = MS response of target compound/ MS response of internal standard; x = [compound]/ [internal standard].

Table 2.3 Regression equations for milk flavor compounds analyzed by SBSE-GC-MS method

Compounds	Quantify ion	Qualify ion	Internal standard	Regression equation[1]	R^2
2-heptanone	43	58, 71	3-heptanone	y=0.8889x	0.9810
2-nonanone	58	43, 71		y=0.8584x	0.9844
2-undecanone	58	43, 71		y=0.6184x	0.9819
γ-nonalactone	85	56, 100	Ethyl cinnamate	y=0.9491x	0.9948
γ-decalactone	85	128, 55		y=1.046 x	0.9930
γ-dodecalactone	85	55, 128		y=0.3845x	0.9853
δ-decalactone	99	71, 55		y=0.4203x	0.9808
δ -undecalactone	99	71, 55		y=0.4699x	0.9876
δ -dodecalactone	99	71, 114		y=0.2026x	0.9855

[1]y = MS response of target compound/ MS response of internal standard; x = [compound]/[internal standard].

Table 2.4 Concentrations (μg/L) of volatile compounds in UHT milk samples at 1 week

Compounds	Plate	Ohmic	Steam	Pasteurized
Sulfur compounds				
DMS	25±3 a[a]	11±0.5 b	0.4±0.2 c	5.4±0.2 d
MeSH	13±1 a	13±1 a	12±4 a	nd[b]
DMDS	0.7±0.5 a	0.7±0.6 a	0.9±0.8 a	nd
DMTS	0.3±0.2 a	0.2±0.1 a	0.3±0.2 a	nd
Methyl ketones				
2-heptanone	51±7 a	17 ±4 b	12±2 b	8±1 c
2-nonanone	41±5 a	10±1 b	9±1 b	5±1 c
2-undecanone	42±8 a	13±2 b	10±2 b	2.2±0.2 c
Lactones				
γ-nonalactone	0.5±0.1 a	0.6±0.2 a	0.8±0.1 a	0.3±0.03 b
γ-decalactone	1.1±0.2 a	1.2±0.1 a	1.5±0.3 a	0.4±0.03 b
γ-dodecalactone	38±2 a	37±5 a	34±11 a	9±1 b
δ-decalactone	143±11 a	147±7 a	186±20 b	62±2 c
δ -undecalactone	5.2±0.8 a	5.0±1.0 a	4.4±0.8 a	1.6±0.1 b
δ -dodecalactone	448±94 a	431±97 a	521±138a	132±7b

Table 2.4 Concentrations (µg/L) of volatile compounds in UHT milk samples at 1 week (Continued)

Compounds	Plate	Ohmic	Steam	Pasteurized
Aldehydes				
2-methylpropanal	55±13 a	103±32 b	29±9 a	nd
Benzaldehyde	6.0±0.7 a	2.7±0.6 a	4.5±2.6 a	nd
Hexanal	31±14 a	43±5 a	145±15 b	7±3 c
Heptanal	5.9±1.6 a	5.4±1.0 a	6.4±0.1 a	0.8±0.2 b
Octanal	1.0±0.3 a	1.1±0.3 a	1.4±0.0 a	nd
Nonanal	3.6±0.9 a	3.6±1.5 a	3.2±2.1 a	nd

[a] Different letters for the same compound indicate significant difference between sample means (Tukey HSD 95%, standard deviations were calculated from six replicates).

[b] Below detection limit.

Table 2.5 Concentrations (μg/L) of volatile compounds in UHT milk samples during storage from 4-36 week

Compounds	4w			16w			36w		
	Plate	Ohmic	Steam	Plate	Ohmic	Steam	Plate	Ohmic	Steam
Sulfur compounds									
DMS	21±1 a[a]	10±1 b	0.8±0.1 c	21±6 a	9±2 b	2±0.5 c	18±4 a	11±3 b	5±2 c
MeSH	10±4 a	11±5 a	10±2 a	8±4 a	5±3 a	9±4 a	5±3 a	7±3 a	6±4 a
DMDS	1.3±0.4 a	1.2±0.4 a	1.1±0.4 a	0.9±0.4ab	0.4±0.3 a	1.0±0.5 b	0.6±0.2 a	0.4±0.1 b	0.5±0.2 c
DMTS	0.4±0.2 a	0.2±0.04 a	0.2±0.16 a	0.15±0.1 a	nd[b]	0.3±0.2 a	nd	nd	nd
Methyl ketones									
2-heptanone	60±5 a	19±1 b	12±1 b	58±7 a	19±3 b	14±3 b	61±12 a	30±2 b	28±1 b
2-nonanone	47±5 a	15±3 b	8±1 c	47±6 a	14±2 b	10±2 b	49±11 a	22±2 b	22±1 b
2-undecanone	44±9 a	16±7 b	11±1 b	56±13 a	18±5 b	11±1 b	55±14 a	28±5 b	28±2 b
Lactones									
γ-nonalactone	1.1±0.2 a	0.7±0.2 b	0.9±0.2ab	1.3±0.4 a	1.6±0.5 a	1.0±0.1 a	1.3±0.1 a	1.1±0.1 a	1.1±0.4 a
γ-decalactone	1.7±0.4 a	1.8±0.4 a	2.0±0.3 a	3.4±0.7 a	3.3±0.2 a	2.5±0.3 b	3.2±0.3 a	3.0±0.4ab	2.6±0.4 b
γ-dodecalactone	60±15 a	109±24 b	63±4 a	167±52 a	123±19ab	99±22 b	170±65 a	153±49 a	127±53 a
δ-decalactone	186±21ab	226±42 a	180±12 b	87±13 a	53±25 b	56±6 b	14±7 a	11±2 a	11±0 a
δ-undecalactone	7.9±2.4ab	9.8±2.0 a	5.4±1.7 b	5.3±0.9 a	3.0±1.6 b	2.6±0.6 b	1.2±0.4 a	0.9±0.4 a	1.2±0.5 a
δ-dodecalactone	632±161ab	801±125a	528±75 b	567±100a	286±122b	266±28 b	213±84 a	156±62 a	158±47 a

Table 2.5 Concentrations (µg/L) of volatile compounds in UHT milk samples during storage from 4-36 week (Continued)

Aldehydes									
2-methylpropanal	63±24 a	130±28 b	50±14 a	96±35 a	92±35 a	30±14 b	138±55 a	29±10 b	38±12 b
Benzaldehyde	6.2±0.6 a	4.9±0.7 a	6.5±2.1 a	9±2 a	8±3 a	11±4 a	19±6 a	10±4 a	18±3 a
Hexanal	57±25 a	89±20 a	80±29 a	27±10 a	38±12 a	36±12 a	70±2 a	20±1 b	18±6 b
Heptanal	6.6±1.0 a	7.0±0.8 a	7.0±3.2 a	6.7±1.6 a	10.3±2.9a	8.5±4.0 a	13.9±2.1a	4.3±0.5 b	6.2±2.4 b
Octanal	1.1±0.2 a	1.1±0.2 a	1.2±0.1 a	0.4±0.1 a	0.5±0.2 a	0.6±0.1 a	0.5±0.2 a	nd	nd
Nonanal	2.8±1.0 a	2.5±1.1 a	2.6±0.5 a	1.2±0.6ab	0.7±0.4 a	2.3±0.3 b	0.4±0.3 a	1.8±0.5 b	nd

[a] Different letters for the same compound indicate significant difference between sample means (Tukey HSD 95%, standard deviations were calculated from six replicates).

[b] Below detection limit.

Table 2.6 Odor activity values (concentration/reported threshold) for volatile compounds in UHT milk samples

Compounds	Aroma Threshold[1] (μg/kg)	Pasteurized	1w			4w			16w			36w		
			Plate	Ohmic	Steam	Plate	Ohmic	Steam	Plate	Ohmic	Steam	Plate	Ohmic	Steam
Sulfur compounds														
DMS	2	2.7	12.3	5.5	0.2	10.4	4.9	0.4	10.2	4.5	1.1	9.1	5.6	2.4
MeSH	0.2	nd[a]	67	64	62	51	52	48	42	27	42	26	35	31
DMDS	0.16	nd	4.5	4.6	5.4	8.4	7.2	6.8	5.5	2.7	6.2	4.0	2.8	3.3
DMTS	0.008	nd	36	26	31	55	25	27	18	nd	39	nd	nd	nd
Methyl ketones														
2-heptanone	5	1.6	10.2	3.4	2.4	12.0	3.8	2.4	11.6	3.8	2.8	12.2	6.0	5.6
2-nonanone	5	1	8.2	2.0	1.8	9.4	3.0	1.6	9.4	2.8	2.0	9.8	4.4	4.4
2-undecanone	7	0.3	6.0	1.9	1.4	6.3	2.3	1.6	8.0	2.6	1.6	7.9	4.0	4.0
Lactones														
γ-nonalactone	65	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
γ-decalactone	5	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
γ-dodecalactone	7	1.3	5.4	5.3	4.9	8.6	15.6	9.0	23.9	17.6	14.1	24.3	21.9	18.1

Table 2.6 Odor activity values (concentration/reported threshold) for volatile compounds in UHT milk samples (Continued)

Compounds	Aroma Threshold[1] (µg/kg)	1w				4w			16w			36w		
		Pasteurized	Plate	Ohmic	Steam	Plate	Ohmic	Steam	Plate	Ohmic	Steam	Plate	Ohmic	Steam
δ-decalactone	100	0.6	1.4	1.5	1.9	1.9	2.3	1.8	0.9	0.5	0.6	0.1	0.1	0.1
δ -undecalactone	150	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
δ -dodecalactone	100	1.3	4.5	4.3	5.2	6.3	8.0	5.3	5.7	2.9	2.7	2.1	1.6	1.6
Aldehydes														
2-methylpropanal	0.7	nd	79	147	41	90	186	71	137	131	43	197	41	54
Benzaldehyde	350	nd	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Hexanal	4.5	1.6	6.9	9.6	32.2	12.7	19.8	17.8	6.0	8.4	8.0	15.6	4.4	4.0
Heptanal	3	0.26	2.0	1.8	2.1	2.2	2.3	2.3	2.2	3.4	2.8	4.6	1.4	2.1
Octanal	0.7	nd	1.4	1.6	2.0	1.6	1.6	1.7	0.6	0.7	0.9	0.7	nd	nd
Nonanal	1	nd	3.6	3.6	3.2	2.8	2.5	2.6	1.2	0.7	2.3	0.4	1.8	nd

[1]Values measured in water.(Buttery et al., 1988, Rychlik et al., 1998, Siek et al., 1971).
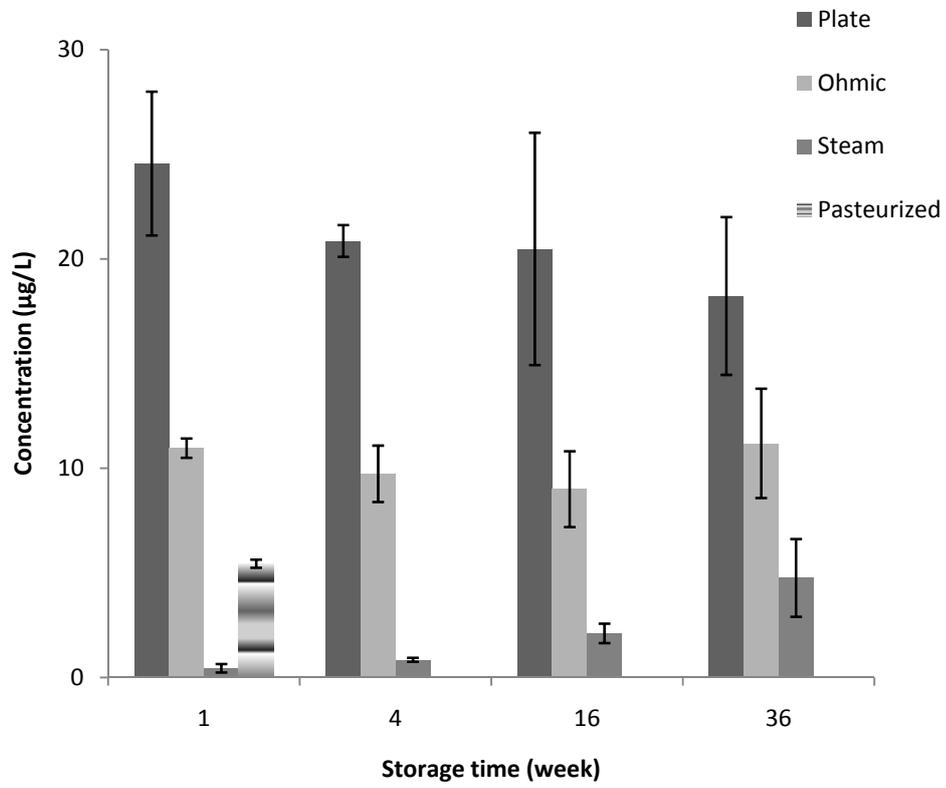
[a] Below detection limit.

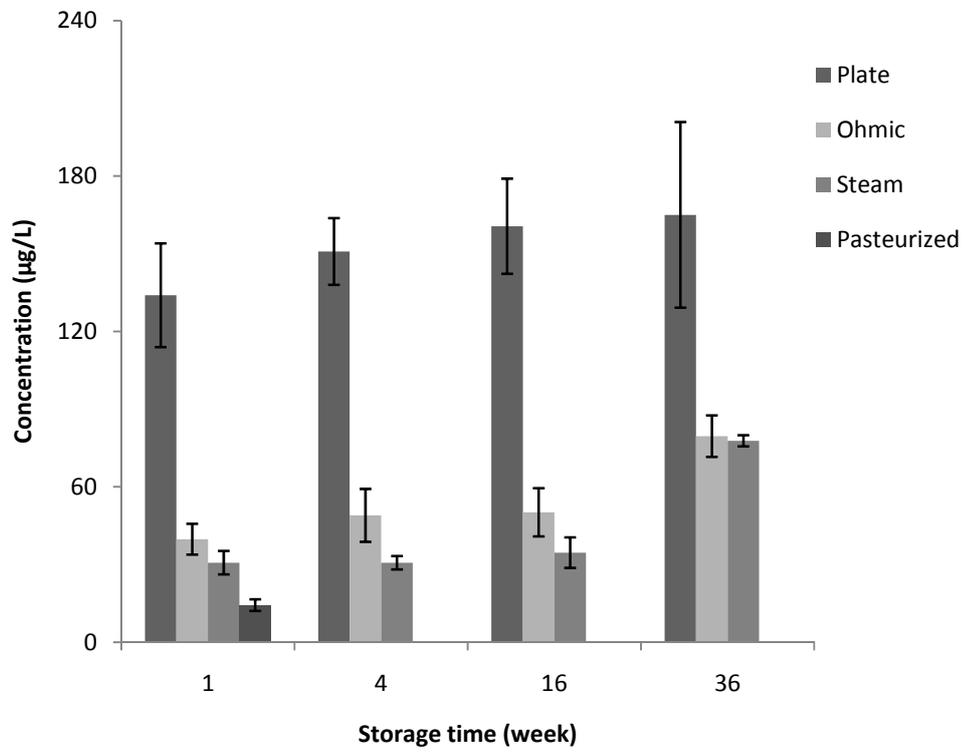**Figure 2.1** Concentration of DMS in milk samples during storage

Figure 2.2 Concentration of total ketones in milk samples during storage
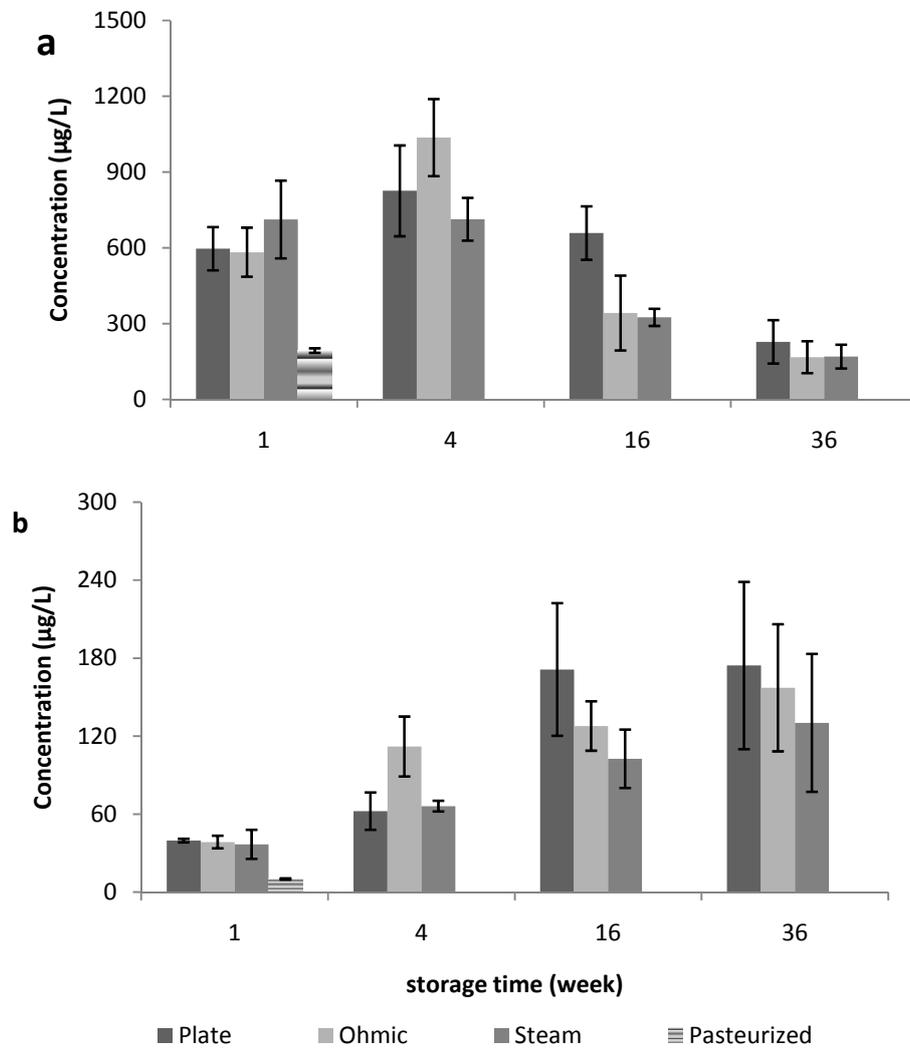
Figure 2.3 Concentration of volatile compounds in milk samples during storage: a) total delta lactones; b) total gamma lactones

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Chapter 3 AROMA RECOMBINATION OF KEY ODORANTS IN ULTRA-PASTEURIZED MILK

3.1 Abstract

Application of aroma recombination experiment was applied on key odorants, including methyl ketones, lactones, aldehydes and sulfur-containing compounds in ultra-pasteurized (UP) milk. The overall aroma of the UP milk could be mimicked by recombining 15 reference odorants in the same concentrations as they occurred in the ultra high temperature (UHT) milk using commercial pasteurized milk as the matrix, which indicated that these four groups of compounds contributed the overall UHT milk flavor.

Key words: UP milk, aroma, and aroma recombination.

3.2 Introduction

During the first period of aroma-related research, flavor analysis was performed under the assumption that all the volatiles occurring in food contributed to its overall aroma. Therefore, the analytical procedure, normally a combination of gas chromatography and followed detector, mostly mass spectrometry, was confined to the identification of the volatiles showing up as peaks in the gas chromatogram.

However, the controversy was raised by Rijkens and Boelens in 1975 that whether all these volatiles actually contributed to the aromas (Grosch, 2001). The doubt encouraged further study to calculate odor activity values (OAVs) of volatile compounds. New method for flavor research began to combine objective measurements (e.g. instrumental analysis of volatile compounds) and subjective measurements (e.g. sensory analysis). Consequently, techniques were focused more on the identification of compounds with higher OAVs contributing to the aroma and the correlation on sensory properties and analytical results. Furthermore, the conclusion was summarized that less than 5% of the volatiles identified in foods contributed to their overall aromas (Grosch, 2000).

In order to prove analytical results obtained by flavor chemists were correct, synthetic mixtures containing odorants were prepared on the basis of the obtained analytical data and their aromas were compared with those of the originals, which termed "aroma recombination" experiments.

The aim of this study was to perform the aroma recombination test, as well as the supporting sensory evaluations to testify the importance of aroma compounds measured in analytical procedure above.

3.3 Materials and methods

3.3.1 Experimental Design

Due to the limitation of additional UHT milk sample received from Utah State

University and the depletion of commercial UHT milk from local store, UP milk was

used in this sensory evaluation study. Since the only difference between UP and

UHT milk is that there is no aseptic packaging in UP milk (Dunkley and Stevenson,

1987). It's assumed that the UP would reflect similar heat effects as UHT milk. 2%

pasteurized milk and UP milk were obtained locally from Fred meyer branded

"Organic Valley".

Four important groups of aroma-active compounds (methyl ketones, lactones,

aldehydes and sulfur compounds) were chosen based on the aroma contribution

and reported OAVs above. DMS was separated from other sulfur compounds and

added into pasteurized milk alone due to its important aroma contribution. 2%

commercial pasteurized milk was used as the matrix for its mild aroma perception

and very similar constituent, in which the aroma-active compounds were

dissolved.

The sensory evaluation of the aroma models and the commercial ultra pasteurized

milk was performed by 8 panelists from Food Science and Technology department,

Oregon State University. The assessors were subjected to a ranking test with a

series of five different levels of each model (**Table 3.1**) and were asked to choose one level that was most similar to commercial ultra pasteurized milk for each model. The stock solutions of all odorants selected in these experiments were prepared in ethanol for each aroma model.

The evaluation of the odor (orthonasal) was performed by asking the panelists to evaluate the intensities of the seven odor qualities represented by the mixed chemicals given above at the concentration listed in **Table 3.2**, as well as the control sample and matrix using the six point linear scale of 0, 1, 2, 3, 4 and 5. The results obtained were averaged for each odor note and plotted in a spider web diagram. Sensory analyses were performed in a sensory panel room at 23 ± 1 ^{o}C.

3.3.2 Chemicals

Methanethiol (MeSH), dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), 2-methylpropanal, hexanal, heptanal, nonanal, 2-heptanone, 2-nonanone, 2-undecanone, γ-dodecalactone and δ-decalactone were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dimethyl sulfide (DMS), δ–dodecalactone were supplied by TCI America (Portland, OR, USA). Benzaldehyde was bought from Polyscience Corp (Niles, IL). Ethanol (pure) was obtained from Koptec (King of Prussia, PA).

3.4 Results and conclusions

3.4.1 Ranking test

The sensory panel evaluated either the preference of different levels or the similarity of the overall aroma in comparison to the ultra pasteurized milk for each aroma model solution. For most model solutions, the panelists cannot conclude an agreement on the preference order, which indicated that these groups at each five levels were still within the range of an acceptable milk aroma concentration and contributed milk flavor positively in this range. The agreement was reached only for aldehyde group (model III) that all panelists preferred the least concentrations in milk, which showed that the aldehyde group gave a negative odor impact at these levels.

The aroma attributes of these selected compounds were reported in previous studies (**Table 3.3**). The sensory test provided additional information of odor quality for each group of compounds in this study. All these groups of compounds played very important roles on the overall UHT milk flavor, even at lowest concentration. Methyl ketones contributed creamy and cooked flavor at low concentration, and gave whey flavor when concentration higher than level 4. Lactones gave peachy flavor more than creamy, cooked and cocoanut aroma when concentration higher than level 3, which was not identical with normal lactones flavor contribution. However, it might due to the lack of short chain

lactones. Aldehydes gave green, fresh milk aroma at very low level, and showed

higher intensity of rancid, oxidized off-aroma when concentration increased. DMS

attributed desirable aroma of fresh raw milk at low concentration, and began

offering sweet and slightly cooked aroma when concentration went higher. Only

until the concentration reached level 5, aroma that strong cooked but not

cabbage-like domain. Other sulfur compounds gave cooked aroma when

concentrations were low, but provided cabbage-like off flavor at higher levels, 4

and 5.

The different flavor notes were observed when compared with information

showed in **Table 3.3**. The main cause of this difference might be the matrix effect,

since the results in earlier studies were normally collected in water or oil matrix,

which had a totally different constituent profile when compared with milk. Other

reasons including aroma interaction which had impact on sensory thresholds and

the different heating processing may also affect the aroma perception.

The most similar level of each model picked out by panelists was in accord with

the concentrations reported in Chapter 2, by which the concentrations used to

spike in the aroma recombination experiment was determined (**Table 3.2**).

3.4.2 Aroma recombination experiment

The odor and aroma intensities of "creamy", "cooked milk", "sweet" and "oxidized", "fruity" and "sulfur" were evaluated by the sensory panel. The aroma model containing all groups of odorants listed in **Table 3.2** revealed a good similarity to the aroma of the commercial ultra pasteurized milk. Furthermore, the aroma profile analysis showed that both the ultra pasteurized milk and the recombinate elicited the same intensities for the odor qualities creamy, sweet and sour and nearly identical intensities for the odor qualities cooked and oxidized (**Figure 3.1**). Only the odor descriptors- fruity and sulfur was rated lower in the ultra pasteurized milk compared to the model solution. The difference might come from the difference between the commercial ultra pasteurized milk and the UHT milk analyzed in this study, or the flavor interactions since the concentrations of each model were determined respectively.

Table 3.1 Concentrations of aroma active compounds for each model

Models	Compounds	Concentrations in milk (µg/L)				
		Level 1	Level 2	Level 3	Level 4	Level 5
Model I	2-heptanone	7	33	65	130	326
	2-nonanone	6	28	56	112	279
	2-undecanone	5	24	47	95	236
Model II	γ-dodecalactone	11	54	107	214	536
	δ-decalactone	20	101	202	404	1009
	δ –dodecalactone	84	420	841	1682	4205
Model III	2-methylpropanal	12	61	122	244	610
	Benzaldehyde	2	8	17	33	83
	Hexanal	15	73	146	292	730
	Heptanal	1	5	10	20	49
	Nonanal	0.3	1.4	2.9	5.8	14.4
Model IV	MeSH	0.92	4.6	9.2	18.3	46
	DMDS	0.11	0.53	1.1	2.1	5.3
	DMTS	0.02	0.09	0.19	0.37	0.93
Model V	DMS	2	10	20	40	100

Table 3.2 Concentrations of aroma active compounds for aroma recombination experiment

Compounds	Spiked concentrations in milk (µg/L)
2-heptanone	65
2-nonanone	56
2-undecanone	47
γ-dodecalactone	107
δ-decalactone	202
δ –dodecalactone	841
2-methylpropanal	61
Benzaldehyde	8
Hexanal	73
Heptanal	5
Nonanal	1.4
MeSH	9.2
DMDS	1.1
DMTS	0.19
DMS	20

Table 3.3 Aroma attributes and sensory thresholds of volatile compounds

| Compound	Aroma attributes	Literature sources
2-heptanone	Blue cheese, fruity, sweet	(Buttery et al., 1988, Karahadian et al., 1985, Preininger and Grosch, 1994, Siek et al., 1969)
2-nonanone	fruity, musty, rose, tea-like	
2-undecanone	floral, herbaceous, fruity	
γ-dodecalactone	peach, butter, sweet, floral	(Engel et al., 1988, Preininger and Grosch, 1994, Schieberle et al., 1993, Siek et al., 1971, Urbach et al., 1972)
δ-decalactone	coconut, apricot	
δ-dodecalactone	fresh fruit, peach	
2-methylpropanal	malty, cocoa, green, pungent	(Ahmed et al., 1978, Amoore et al., 1976, Guadagni et al., 1972, Larsen and Poll, 1992, Siek et al., 1971)
Hexanal	grassy, green, tallow	
Heptanal	fatty, green, woody, fruity	
Nonanal	citrus, green, fatty, floral	
DMS	Uncleaned, sulfur-like cabbage	(Badings and de Jong, 1984, Rychlik et al., 1998)
MeSH	unpleasant cabbage, sulfur-like cabbage	(Rychlik et al., 1998)
DMDS		
DMTS		
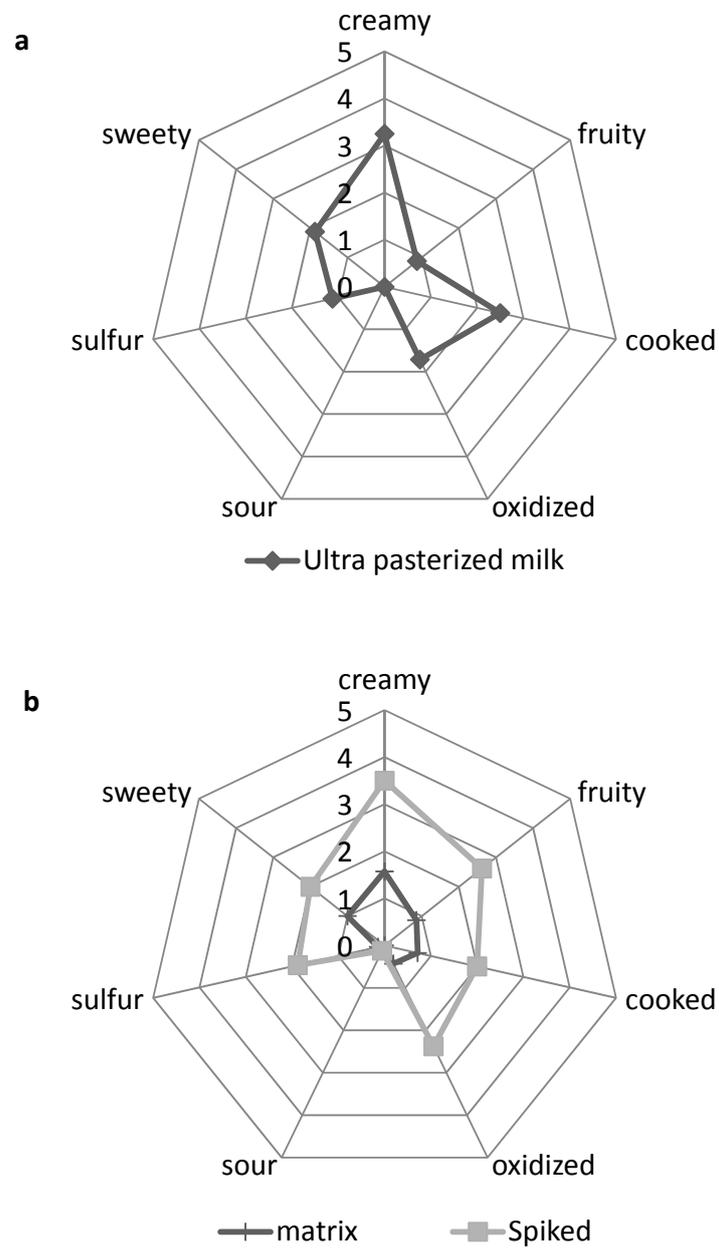
Figure 3.1 Comparative aroma profiles of a) the ultra pasteurized milk; b) the aroma model mixture and the matrix

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Chapter 4 CONCLUSION

Volatile analysis in different types of UHT milk indicated that sulfur compounds, methyl ketones, aldehydes, and lactones were the most important aroma compounds for UHT milk flavor. These groups of compounds were quantitatively, but not qualitatively different for three UHT milk, including Ohmic heating, direct steam injection and indirect plate heating UHT milk. A thoroughly volatile comparison between these UHT milk showed methyl ketones and DMS were highly related to the severity of heating applied to milk, which had highest concentration in indirect plate heating milk due to higher temperature generated at the surface between plate exchanger and milk, while lowest amount in direct steam milk caused by flavor loss during the following evaporative cooling. γ-lactones showed higher concentration in Ohmic heating which may related to the optimum "heated" flavor. Aldehydes had higher amount in indirect plate heating milk at later stage of the shelf life, which associated with "stale" and "rancid" oxidized off-flavor.

The overall flavor development pattern followed UHT milk flavor generation trend. Sulfur compounds that responsible for the cooked flavor decreased during storage. γ-lactones developed during the first month, considered optimum intermediate heated flavor of UHT milk. And aldehydes were found increasing trend in heptanal and strecker aldehydes, hexanal, octanal and nonanal decreased during storage. Elevated

stale flavor perception may be related to the decrease in threshold levels for

aldehydes as lactone levels decreased during storage.

A further study moved to the flavor recombination test, which gave an overall

similarity of Ultra pasteurized milk except "fruity" notes. The results confirmed the

importance of these aroma compounds in UHT milk.

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