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Factors affecting the flavor development of Swiss cheese

by

Firth Kraft Whitehouse

A Dissertation Submitted to the

Graduate Faculty in Partial Fulfillment of the

Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Department: Food Science and Human Nutrition
Major: Food Science and Technology

Approved:

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In Charge of Major Work

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Iowa State University
Ames, Iowa

1995

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DEDICATON

This dissertation is dedicated to the memory of my grandfather

John "Ton" Ferris

1903- 1993

He was the first person in our family to receive a college degree

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INTRODUCTION

People in the United States have become increasingly health-conscious over the last decade, resulting in a desire to avoid high-fat foods, reduce dietary fat intake, and monitor the consumption of saturated fatty acids and cholesterol. In addition, some consumers must limit their intake of saturated fat and cholesterol for specific health reasons. The food industry has responded by introducing a wide variety of low-fat and fat-free foods (Kennedy, 1991). Many traditional cheeses are rich in fat, and are a major source of saturated fatty acids and cholesterol in U.S. diets. Because of this, there has been great interest in the development of low-fat and fat-free cheeses. However, many of these commercially available products, especially low-fat and fat-free ripened cheeses, are lacking in good flavor, texture, and body.

With such an emphasis on decreased dietary fat intake, the no-fat and low-fat food fad that is currently sweeping the United States has resulted in notions that affect the consumer perception of dairy products, especially cheeses. Because so many of the low-fat and fat-free cheeses have poor flavor, the assumption is made that it is not possible to produce high quality reduced-fat cheeses. Consumers also tend to forget that a low-fat diet is not necessarily a healthful diet; nutrients such as fat-soluble vitamins and essential fatty acids are eliminated from the diet when fat is eliminated. Dairy products are a source of these nutrients, as long as there is some appreciable fat content. To expand the market for altered fat cheeses, and gain consumer acceptance in terms of flavor quality, the flavor of these cheeses must better simulate that of traditional, full-fat ripened cheeses.

Cheeses that are lower in saturated fats and cholesterol can be produced by partially or fully substituting vegetable oil for milk fat. When this is done, the cheese is classified as a filled cheese. Because milk fat in cheese is believed to be a

source of flavorful short-chain fatty acids, as well as a reservoir for various fat-soluble flavor compounds, inclusion of milk fat or flavorful milk fat fractions in filled cheeses would improve their flavor.

This study reports on the results obtained when Swiss cheese was produced from ordinary milk, from isolated milk fat emulsified into skim milk in various ways, and from high-oleate sunflower oil, both with or without the incorporation of natural or synthetic short-chain fatty acids. Fats made from the chemical combination of fats of different origin are called structured lipids. The structured lipids used in this study were produced by interesterifying short-chain triglycerides into high-oleate sunflower so that the percentage of short-chain fatty acids in the final products was similar to the percentage normally found in milk fat. Homogenization is necessary for successful emulsification of the structured lipids with the skim milk.

Chemical, physical, and sensory parameters of the various cheeses were evaluated to determine the effect of the treatments on cheese quality and flavor. A model was developed in which the chemical and physical parameters of cheeses made this way can be used to describe sensory evaluation scores for cheese taste and flavor.

LITERATURE REVIEW

The unique flavor of Swiss cheese has been the topic of numerous studies. Early work by Babel and Hammer (1939) showed that propionic and acetic acids and their salts were important in good flavor development. Since then, a variety of compounds, both water-soluble and fat-soluble, have been shown to be important in Swiss flavor. Hintz et al. (1956) showed a relationship between proline and propionic acid in the sweet flavor of Swiss cheese. Flavor compounds such as aldehydes, amines, alcohols, ketones, carbonyls, organic acids, esters, sulfides, and amino acids were identified in Swiss cheese by Langler et al. (1967). Many investigators have observed a relationship between good flavor and the short-chain free fatty acid (SCFA) content in a variety of cheeses (Langler and Day, 1966; Kristoffersen et al., 1967; Langsrud and Reinbold, 1973; Harper et al. 1978; Biede, 1977; Biede and Hammond, 1979a, 1979b; Mitchell, 1981; Aston and Dulley, 1982; Vangtal and Hammond, 1986; Lobo di Palma, 1987; Ha and Lindsay, 1991). Kowalewska et al. (1985) showed that amino acid-carbonyl complexes were important in Swiss cheese flavor. Still, the exact nature of Swiss cheese flavor has not been completely elucidated.

Alternate Fat Sources in Cheesemaking

Fats are involved in many aspects relating to flavor perception, including mouthfeel, release of flavor components such as fatty acids, and masking of off-flavors. Fats also serve as sources for flavor precursors (Hatchwell, 1994). When the fat content or profile of a food is altered, flavor is usually affected. There have been several attempts to produce cheese in which the natural milk fat has been

replaced by other fats. Kristoffersen et al. (1971) made Cheddar cheeses from 85% soy/15% cottonseed oil or hydrogenated soy oil. Although these cheeses developed some Cheddar-type flavor, control cheeses made with recombined milk fat had more acceptable flavor. Cheddar cheese was also made using sunflower oil, but the curd slurry had strong cereal-type flavors, so ripened cheese was never made with this oil. Attempts by the same authors to make Romano-type cheeses using the same vegetable oils resulted in poor, unacceptable flavor, due primarily to the lack of SCFA in the vegetable oils. Cheddar cheeses made by Kristoffersen et al. (1971) had poor, crumbly texture, which was attributed to the homogenization step that was necessary to emulsify the skim milk with the oils.

Foda et al. (1974) produced Cheddar cheese from natural milk emulsion, or from skim milk that was emulsified by homogenization with buttermilk solids, milk fat globule membrane (MFGM), heated MFGM, or gum acacia and any of the following oils: mineral oil, mineral oil plus dimethyl sulfide, isolated milk fat, deodorized isolated milk fat, deodorized milk fat plus deodorized distillate, deodorized milk fat plus dimethyl sulfide, SCFA-modified Kaola®, unmodified Kaola® (Durkee Foods), or Kaomel® (Durkee Foods). All of these cheeses had a fairly reasonable flavor, however, cheeses made with the natural milk emulsion had superior flavor. The use of gum acacia as an emulsifying agent improved the flavor intensity of the recombined cheeses. Removal of ketone and lactone precursors from isolated milk fat by deodorization did not significantly affect the flavor of the cheese. Foda et al. could not show clearly whether SCFA played a role in the flavor development.

Kubota and Tateishi (1975) produced a "cheese-like fermented food" from synthetic fats made by interesterifying C6:0 - C12:0 even normal-chain fatty acids into animal fats and/or saturated vegetable oil. Traditional cheesemaking methods were used, but were poorly described. Flavors of the resulting cheeses were reported to possess "Italian-," "fruity-," or "cottage cheese-" type qualities. The variations in flavor were attributed to different proportions of SCFA used in fat synthesis.

Johnson (1991) made Swiss cheese by emulsifying isolated milk fat, corn oil, or modified corn oil into skim milk with or without the use of gum acacia or lecithin as emulsifying agents. The corn oil was modified by the interesterification of synthetic SCFA in amounts similar to those found in milk fat. Results showed that cheeses made from the natural milk emulsion had the most acceptable flavor, but incorporation of SFCA into the corn oil improved the flavor over that of cheeses made from unmodified corn oil. Furthermore, cheeses made with gum acacia as an emulsifying agent had improved flavor.

Babayan and Rosenau (1991) summarized the process for making processed cheeses with medium-chain triglycerides (MCT) as the fat source. The MCTs have been used as a replacement for natural triglycerides in diets consumed by individuals who suffer from malabsorption syndromes (Bach and Babayan 1982). In this cheesemaking process, MCTs are combined with acid-coagulated skim milk curds. Natural cheese such as Cheddar or Fontinella is added, along with other proprietary flavor ingredients, and the final MCT content was 30%. The final products have acceptable flavor, and provide reasonable dietary alternatives for malabsorption syndrome-afflicted people. This approach to cheesemaking using altered fats is different from those mentioned above, as the end-product is made for therapeutic uses.

Recent efforts at Nabisco Foods Group to develop alternative synthetic fats have resulted in the invention of SALATRIM®. This group of structured synthetic triglycerides is composed of long-chain saturated fatty acids and SCFA esterified in a variety of ratios to the glycerol backbone (Smith et al., 1994). Synthesis of these triglycerides involves random interesterification of hydrogenated vegetable oils (soy oil and/or canola oil) with triacetin, tripropionin, and/or tributyrin (Klemann et al., 1994; Softly et al., 1994). The resulting triglycerides are inherently lower in caloric value than are natural triglycerides because of the presence of many SCFAs and the poorly digested stearic acid. Recent reports indicated that cheese was made using SALATRIM (Leveille, 1995), but few details regarding cheese processing,

chemical analyses or sensory evaluation were given, presumably because of the proprietary nature of the information.

Emulsifiers Used To Make Recombined Cheeses

Several emulsifying agents have been used in the production of cheese from recombined fat and skim milk. Dairy-derived emulsifying agents include buttermilk solids and isolated MFGM. The MFGM is a thin, complex membrane that surrounds the milk fat globules in normal milk. It is composed primarily of proteins, phospholipids, glycoproteins, triglycerides, cholesterol, and enzymes. The MFGM enables the milk fat to remain dispersed throughout the skim milk (McPherson and Kitchen, 1983). Isolated MFGM is typically obtained from buttermilk by centrifugation and washing (Kanno et al., 1991; Oehlmann et al., 1994). Buttermilk solids are the water-soluble dry matter of buttermilk, including lactose and various proteins.

Vegetable-derived emulsifying agents include lecithin and gum acacia. Lecithin is a phospholipid that is commercially produced as a byproduct of the vegetable oil refining industry. Lecithin is also found in animal products such as egg yolk, but this source is not widely used in the production of emulsifiers. Gum acacia, or gum arabic, is the dried exudate obtained from branches of *Acacia senegal* trees (Osman et al., 1993). This approved food additive is composed of three fractions, including an arabinogalactan fraction, an arabinogalactan-protein complex, and a glycoprotein fraction. The arabinogalactan and arabinogalactan-protein fractions are rich in hydroxyproline and serine residues (Qi et al., 1991), whereas the glycoprotein is rich in aspartic acid (Osman et al., 1993).

Flavor Analysis Of Swiss Cheese

Fractionation Of Flavor Components in Swiss Cheese

Biede and Hammond (1979a) identified three major flavor compound-containing fractions in Swiss cheese. The first fraction contained the water-soluble volatiles (acetic, propionic, and butyric acids, ammonia, and diacetyl), the second fraction contained the water-soluble non-volatile fractions (amino acids, peptides, lactic acids, and salts), and the third fraction contained the oil-soluble components (SCFA >C4:0, pyrazines, carbonyls, lactones, and phenols). These fractions were then analyzed for flavor attributes by using organoleptic methods. The water-soluble non-volatile fraction revealed a characteristic sweetness associated with Swiss cheese, as well as intense nutty, burned, and bitter flavors. The water-soluble volatile fraction yielded sweet, lipolyzed, and acid flavors. The oil-soluble fraction gave nutty, lipolyzed, and fermented flavor notes (Biede and Hammond, 1979b). This fractionation scheme gives a solid framework for exploring important flavor components in Swiss cheese.

Important Flavor Compounds In Swiss Cheese

Water-soluble volatile SCFA are important in Swiss cheese flavor. Acetic and propionic acids, which are produced by the metabolism of microorganisms, are indicators of proper fermentation in cheese. They are normally present in molar ratios of 2.3:1 propionic:acetic (Langsrud and Reinbold, 1973c). Mitchell (1981) concluded that actual concentrations of propionic and acetic acids were as important as their molar ratios. Butyric acid, which comes from lipolysis of milk triglycerides, provides an important cheese-like flavor note. Too much free butyric acid, however, results in a lipolyzed flavor (Langsrud and Reinbold, 1973c; Duncan

and Christen, 1991). SCFA may be quantifiedby gas chromatography (GC) (Deeth et al., 1983; Ceccon 1991). Diacetyl, which is found in the water-soluble volatile fraction, is responsible for a creamy, buttery flavor note (Langler and Day, 1966), and may be analyzed by using HPLC methods (Bednarski et al., 1989). Dimethyl sulfide is also thought to contribute to the sweet flavor of Swiss cheese (Langler et al., 1967), and has been shown to be produced by *Propionibacterium shermanii* (Keenan and Bills, 1968).

Water-soluble, non-volatile amino acids and small peptides are thought to provide a nutty and brothy flavor in Swiss cheese (Biede, 1977). Larger peptides probably impart a bitter note (Hammond, 1994) in Swiss cheese. This bitterness is thought to be related to the amino acid sequence in the peptides (Adda et al., 1982). Lactic acid imparts an acid, sour taste in cheeses (Hammond, 1994). Free amino acids such as proline (Hintz, 1956), alanine, serine, glycine, and threonine (Langsrud and Reinbold, 1973c) impart sweet flavors to Swiss cheese. Calcium and magnesium ions are associated with amino acids and peptides; their removal by ion-exchange chromatography destroyed the sweetness of the water-soluble, non-volatile fraction of Swiss cheese (Biede and Hammond, 1979a). Certain other amino acids act as flavor precursors in the development of branched-chain (Adda et al., 1982), phenylacetic, and phenylpropionic acids (Vangtal and Hammond, 1986). Amino acids and peptides in cheese may be quantified by using electrophoretic (Harper et al., 1980; Harper and Wang, 1981; Harper et al., 1989), chromatographic (Reps et al., 1987), or spectrophotometric methods (Vangtal, 1986).

Kowalewska et al. (1985) showed that a non-volatile carbonyl/amino acid complex, 2,5-dimethyl-4-hydroxy-3(2H)-furanone, gave burned/caramelized flavor to Swiss cheese. This and other similar compounds were quantified by using various chromatographic methods (Kowalewska et al., 1985). Polymerization of methylglyoxal can also give 2,5-dimethyl-4-hydroxy-3(2H)-furanone (Griffith, 1988). Methylglyoxal, as well as glyoxal and dimethylglyoxal, are metabolic products of *Lactobacillus bulgaricus* (Reps et al., 1987). Griffith and Hammond synthesized a

variety of water-soluble, non-volatile compounds by reacting carbonyls and amino acids under conditions similar to those found in cheese. The reaction products included phenylacetaldehyde, dimethylsulfide, and dimethylpyrazine, to name a few. The authors suggested that many of the compounds they synthesized may be significant in Swiss cheese flavor.

The oil-soluble flavor components of Swiss cheese are diverse. Biede and Hammond (1979a) showed that the acid component of the oil-soluble fraction is important in flavor, becauseneutralization with sodium carbonate reduced odor and flavor intensity. SCFA from C6-C12, including minor odd- and branched-chain fatty acids, may be key elements of flavor, despite their relatively small amounts in cheese (Ha and Lindsay, 1991). Pyrazines, created by the reaction of lysine with α-hydroxyketones, have been identified in cheese (Hammond, 1994), and may be important in Swiss cheese flavor (Biede et al., 1979). Gamma- and delta-lactones have been identified in Swiss cheese (Langler et al., 1967). Methyl ketones from C6 -C10 were also detected in Swiss cheese by Langler et al. (1967). In Swiss cheese, methyl ketones probably come from 3-ketofatty acyl groups, which are present in small quantities in milk fat (Hammond, 1994).

Sensory Analysis Of Flavors In Swiss Cheese

Cheese flavors may be analyzed as individual compounds, as fractions, or in whole cheese. Many of the compounds identified in Swiss cheese can be separated and quantified by GC. Often, these compounds are "sniffed" by several observers as they exit the detection port of the gas chromatograph (Griffith and Hammond, 1989). Aroma descriptors for each compound are agreed upon by the observers, and assigned to each individual compound. Although this method analyzes flavor compounds outside of the context of whole cheese, it is important for defining aroma properties of the individual compounds.

Whole oils can be analyzed organoleptically for flavor compounds in artificial emulsion preparations (Stone and Hammond, 1983). This method can be used to analyze individual natural or synthetic flavor compounds, or mixtures of flavor compounds (Dixon and Hammond, 1984). Mitchell (1981) analyzed added synthetic Swiss-like flavors in a processed cheese base by using trained cheese graders.

The most common method for the flavor evaluation of whole Swiss cheese is by the use of trained sensory panelists (Biede and Hammond, 1979b; Vangtal and Hammond, 1986; Johnson, 1991). Typically, eight or more judges are trained for several days to detect particular flavor notes in the cheese. Results generated by the judges may be analyzed statistically, and can offer a good estimate of consumer acceptability of the cheeses.

Correlations of Chemical and Sensory Data

Several investigators have attempted to correlate chemical/physical data with cheese flavor scores generated by sensory panelists. Vangtal (1986) and Johnson (1991) used multivariate techniques to analyze flavor and chemical/physical data. From these data, models were developed to describe sensory evaluation responses as they are related to the chemical and physical properties of the cheese. Johnson (1991) developed a model using five chemical and physical parameters which described 88% of the variation in the cheese treatments examined in her study. Vangtal's model (1986) included seven independent factors which described 90% of the variance in the data. Using these factors, multiple linear regressions showed that up to 85% of the cheese flavor variability in his study could be accounted for by chemical and physical measurements.

MATERIALS AND METHODS

Preparation of *Lactobacillus bulgaricus* AR2 for Viability Studies and α-Dicarbonyl Determination

Lactobacillus bulgaricus AR2 was obtained from the culture collection of the Department of Food Science and Human Nutrition at Iowa State University. *Lactobacillus bulgaricus* AR2 was grown in 94.8 g/L sterile reconstituted Carnation natural nonfat dry milk (Carnation Co., Los Angeles, CA) at 37°C. Eighteen-hour cultures of *L. bulgaricus* AR2 were frozen in 1.0-mL batches containing equal volumes of glycerol and culture broth, and stored at -80°C until needed. Lactobacilli MRS broth (Difco, Detroit, MI) was prepared according to label instructions using 55.0 g medium/L distilled deionized water. The broth was autoclaved at 121°C, 15 psi for 15 minutes. When a culture was needed for viability and/or dicarbonyl production studies, it was thawed, inoculated at 1% (v/v) into Lactobacilli MRS medium, and incubated for approximately 36 hrs. Two additional transfers at 1% (v/v) of 18-h cultures were made to obtain active cultures. Viable cell counts were determined in duplicate by a pour plate method using Lactobacilli MRS medium containing 2.0% agar. All 18-h cultures contained $2.9x10^9$ - $3.3x10^9$ CFU/mL.

Determination of α-Dicarbonyl Production By *Lactobacillus bulgaricus*

Production of glyoxal, methylglyoxal, and diacetyl by *L. bulgaricus* AR2 was quantified using a high-preformance liquid chromatography (HPLC) method modified from Bednarski et al. (1989). The cultures were adjusted to pH 8.0 with 1 N sodium hydroxide, and centrifuged at 20,000 x *g* for 15 min. In order to quantify the

dicarbonyls using HPLC, they needed to be converted to quinoxaline derivatives. To do this, 1.0 mL of the adjusted culture broth was reacted with 50 µL of a 5.0 mg/mL solution of *o*-phenylenediamine (Sigma Chemical Co., St. Louis, MO) at 25°C for four hours. The sample was then brought to pH 3.0 with 250 µL 1 N hydrochloric acid. To extract the quinoxaline derivatives, the sample was mixed vigorously with 2 mL distilled diethyl ether using a Vortex mixer. The ether layer was recovered following a brief centrifugation, and the extraction was repeated two additional times. The pooled ether extract containing the quinoxaline derivatives was evaporated at 40°C until nearly dry. One mL HPLC grade methanol (Fisher Scientific Co., Pittsburgh, PA) was added to the residue, and the sample was filtered through a 0.45 µm nylon filter (Gelman Scientific, Ann Arbor, MI) prior to HPLC analysis.

A Shimadzu (Kyoto, Japan) HPLC Model LC-600, equipped with a 20 µL fixed-volume injection loop and a Shimadzu SPD-6AV UV/visible variable-wavelength detector, was used to analyze the quinoxaline derivatives of dicarbonyls produced by *L. bulgaricus* AR2. The detector was operated at 315 nm, and output was integrated by a Shimadzu CR501 integrator. Quinoxaline derivatives of dicarbonyls were separated by a Supelcosil LC-18 (Supelco, Bellefonte, PA) column, 250 x 4.6 mm, particle size 5 µm, equipped with a Supelguard LC-18 20 mm guard column (Supelco, Bellefonte, PA). The mobile phase was 70:30 methanol:water, delivered at 0.65 mL/min.

Quinoxaline, methylquinoxaline, and dimethylquinoxaline (Sigma Chemical Co., St. Louis, MO) standards (> 99% purity), ranging in concentration from 10 µg/mL to 400 µg/mL, were prepared daily in methanol, and used to determine standard curves for sample quantitation. A linear response was observed over this range of standard concentrations for each quinoxaline derivative. Dicarbonyl concentrations were calculated by using the standard curve method.

Heat-Shock Treatment of *Lactobacillus bulgaricus* AR2

For each heat shock treatment, 0.5 mL of an 18-h culture of *L. bulgaricus* AR2 was inoculated into three replicate 20-mL culture tubes containing 9.5 mL Lactobacilli MRS broth. The inoculated samples were then incubated for 1.5 h at 32°C. This time and temperature was chosen to mimic those used in cheesemaking once the cultures are added to the milk. Culture tubes were then immersed in a water bath at 55°C, and held until the desired internal temperature of the tubes was reached. A separate tube containing uninoculated broth and a glass thermometer inserted through a rubber septum was used as a crude thermocouple to monitor temperatures inside the tubes. To reach an internal temperature of 42°C, the cultures were heat-shocked for 5 sec. Approximately 40 sec were needed to reach an internal temperature of 50°C.

Following the heat-shock treatment, the culture tubes were incubated at 37°C for up to 72 h. As controls, replicate cultures were prepared in a similar manner, except that they were transferred to a 37°C incubator directly following the 1.5-h, 32°C incubation period.

Viability of *Lactobacillus bulgaricus* AR2 in Oleic Acid-Containing Medium

Sodium oleate was prepared by reacting 1.0 g saturated sodium hydroxide with 1.0 g oleic acid (Sigma Chemical Co., St. Louis, MO), using the method of Anders and Jago (Anders and Jago, 1970b). The reaction mixture was stirred for 30 min., and the resulting powder was ground using a porcelain mortar and pestle. Finally, the ground powder was blotted between layers of No. 4 Whatman filter paper, and dried under ambient conditions.

Five millimolar oleic acid-containing Lactobacilli MRS medium was prepared by dissolving 55.0 g Lactobacilli MRS medium and 5.0 mmoles oleic acid as sodium

oleate in 1 L distilled, deionized water. The medium was autoclaved at 121°C for 15 min. in 20 mL test tubes containing 9.9 mL medium. Final pH of the medium was 6.2. One-tenth milliliter of 18-h culture of *L. bulgaricus* AR2 containing 2.9 x 10^9 cells/mL was inoculated into each of three replicate 20-mL culture tubes containing 9.9 mL oleic acid MRS broth. Control samples were prepared using Lactobacilli MRS broth without 5.0 *mM* oleic acid. Cultures were incubated at 37°C for 24, 48, 72, and 96 h.

α-Dicarbonyl Production By *Lactobacillus bulgaricus* AR2 in Oleic Acid-Containing Medium

Production of α-dicarbonyls by *L. bulgaricus* AR2 grown in 5.0 *mM* oleic acid Lactobacilli MRS broth was determined using previously described methods modified from Bednarski et al. (1989). Samples were taken from culture broth that was incubated for 24, 48, 72 and 96 h.

Preparation of Milk Fat Fatty Acid Methyl Esters

As a first step in the isolation of C4:0-C12:0 fatty acids from milk fat, it was necessary to make fatty acid methyl esters. These methyl esters were used for both analytical and preparative purposes.

USDA Grade AA sweet cream butter (Prize of Iowa, Mid-America Farms, Springfield, MO) was melted in a 600 watt microwave on the "high" setting. The melted butter was strained through three double layers of cheesecloth and centrifuged in 250-mL Nalgene bottles for 20 minutes at 1,900 x *g*. The oil phase was collected, and methyl esters of the fatty acids were prepared. This was achieved by reacting the milk fat for 6 h with a solution of methanol/3% sulfuric acid

under reflux. Three moles of methanol solution per mole of milk fat were used, plus a 5% excess of the methanol solution in order to prevent partial glyceride formation. The methyl esters were washed with distilled water, followed by a 5% aqueous sodium carbonate wash, and a final distilled water wash. The methyl esters were dried over anhydrous sodium sulfate.

The milk fat as methyl esters were separated on a 15-m wide-bore fused silica capillary column (i.d. 0.53 mm) coated with Nukol phase at a film thickness of 0.50 μm (Supelco, Bellefont, PA) using a Varian 3700 gas chromatograph (Varian Assoc., Inc., Sunnyvale, CA). Helium at 15 mL/min was the carrier gas. Both the injection port and the flame ionization detector (FID) were set at 220°C. The column was held at 40°C for two minutes, programmed at a rate of 10°C/min until a temperature of 200°C was reached, and held at 200°C for 10 min. Samples were diluted to approximately 10 mg/ml. Injection volume was 1 μL.

For some samples, further esterification was needed to complete the formation of fatty acid methyl esters. To do this, a solution of 1*M* sodium methoxide in methanol was prepared and added to the sample in a 5% excess of a 3:1 solvent:sample molar ratio. The mixture was refluxed for 1 h, cooled, and neutralized with 5% aqueous acetic acid. The sample was washed twice with distilled water and dried over anhydrous sodium sulfate.

Natural Short-Chain Triglyceride Preparation

Natural short-chain triglycerides (SCTG) were prepared using free fatty acids that were distilled as methyl esters prepared from natural milk fat. The distillation was performed under vacuum using an oil pump (Duo Seal Vacuum Pump, The Welch Scientific Co., Skokie, IL). A Cartesian manostat (The Emil Greiner Co., NY, NY) was used to control the vacuum, which was slowly lowered from 150 mm Hg to 1.0 mm Hg. A detailed diagram of the distillation setup is shown in Figure 1. The

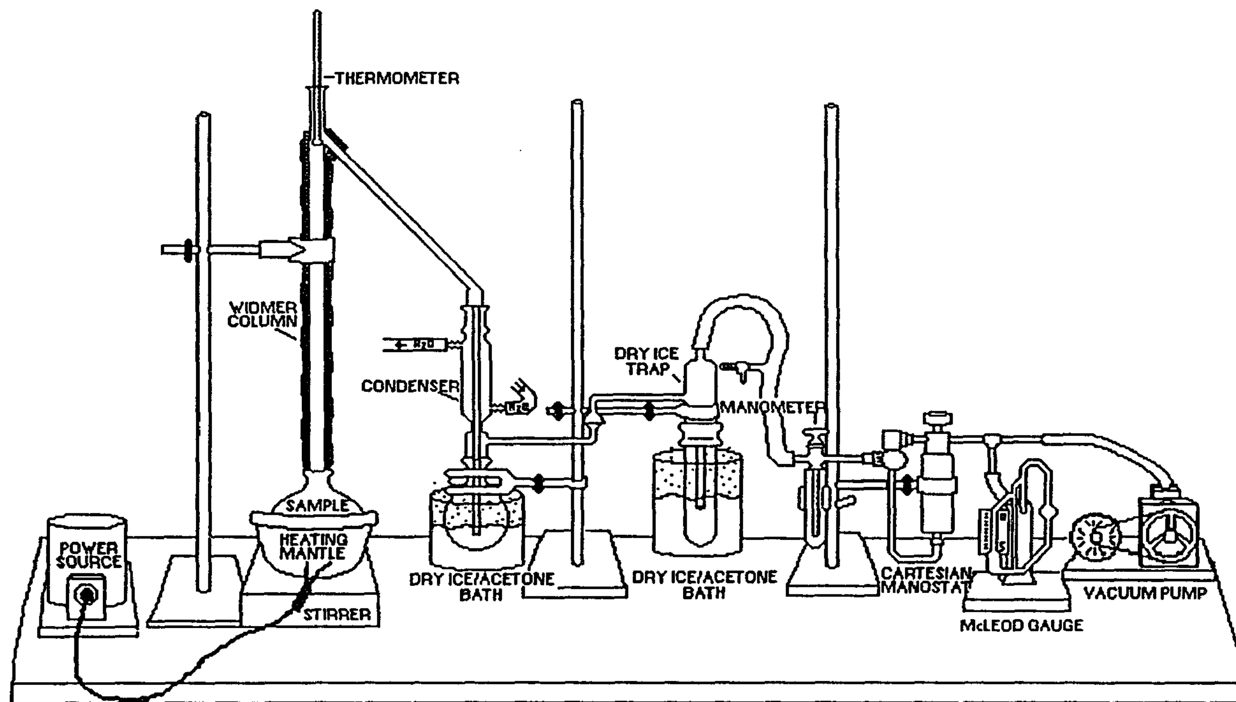

Figure 1. Schematic diagram of FAME distillation apparatus

column head temperature was slowly raised to distill methyl esters up to but not including C14:0. It was necessary to insulate the reaction flask and the 40-cm Widmer column in order to ensure complete distillation of the desired compounds.

A cold saponification procedure described by Markley (1964) was used to liberate the free fatty acids from the methyl esters. This method was chosen to prevent losses of fatty acids that would otherwise be volatilized or lost in the aqueous phase of a standard saponification reaction. In this method, 258 mL of 50% aqueous KOH were added to 253.0 g fatty acid methyl esters. Ethanol (roughly 1% by weight of the methyl esters) was added to catalyze the reaction, and the mixture was stirred vigorously for 15 min. Excess methanol and ethanol were removed using a rotary evaporator at 50°C. The soaps were acidified using concentrated hydrochloric acid, while keeping the reaction flask on ice in order to prevent volatile losses. The free fatty acids were recovered and dried over anhydrous sodium sulfate.

Since several distillations and saponifications were performed, the final free fatty acids from all batches were pooled, and molar quantities of the even n-chain free fatty acids from C4:0 to C12:0 were determined using the GC method described previously.

Short-chain triglycerides (SCTG) were prepared using the benzene azeotrope method of Quinn et al. (1967). The triglycerides were prepared in several batches. Each batch consisted of 6.46 moles of even n-C4:0-C12:0 free fatty acids, 0.10 moles of p-toluenesulfonic acid (Sigma Chemical Co., St. Louis, MO), 2.05 moles of glycerol (Sigma Chemical Co., St. Louis, MO), and 407 mL HPLC Grade benzene (Fisher Scientific, Pittsburg, PA). This mixture included a 5% excess of fatty acids in order to prevent partial glyceride formation. The reaction was refluxed for 20 h, and washed with 5% aqueous sodium carbonate followed by distilled water, to remove unreacted free fatty acids and to neutralize the acid catalyst. The SCTG were dried over anhydrous sodium sulfate, and an Acid Value Test, Method Cd 3a-63, (AOCS,

1990) was conducted for each batch. An Acid Value of less than or equal to 2.0 mg KOH/g sample was considered acceptable.

Synthetic Short-Chain Triglyceride Preparation

Synthetic SCTG were also prepared using commercial fatty acids of > 99% purity (Sigma Chemical Co., St. Louis, MO) by the method of Quinn et al. (1967). The even n-C4:0-C12:0 free fatty acids were used in molar ratios identical to those found in the natural milk fat.

Preparation of the Natural Milk Fat Fatty Acid Emulsion

A natural milk fat fatty acid emulsion was prepared for tasting following the method of Stone and Hammond (1983). Milk fat fatty acids from C4:0-C12:0 were obtained from sweet cream butter as described previously. A fatty acid stock solution was prepared daily by blending 100 μl C4:0-C12:0 natural milk fat fatty acids in 100 ml Sontex 35 white UPS grade mineral oil (Sontex, Dickinson, TX). To prepare the emulsion for tasting, 95 ml tap water, 0.625 g gum acacia (TIC Gums, Bellcamp, MD), and 5 mL fatty acid stock solution were blended for 2 min in a Waring glass blender jar (Waring Prod. Div., Dynamics Corp. of America, New Hartford, CT) using the "liquefy" setting. The mixture was poured into a drinking glass (volume approximately 200 mL), covered with a watch glass, and allowed to sit at 25°C for 15 min before tasting.

Two judges were used for emulsion tasting. Just prior to tasting, each glass containing the emulsion was swirled for several seconds. Then the watch-glass lids were removed, and the samples were smelled and then tasted. The judges held the

emulsions in their mouths for five to ten seconds, allowing the volatiles to fill the oral cavity, before expectorating the sample.

Preparation of Synthetic Fatty Acid Emulsion

The synthetic fatty acid emulsion was prepared as described above, by using n-C4:0-C12:0 fatty acids in molar ratios identical to those of natural milk fat. Tasting was performed as for the natural milk fat fatty acid emulsion.

Preparation of Interesterified Short-Chain Triglycerides and High-Oleate Sunflower Oil

Short-chain triglycerides from natural or synthetic sources were interesterified with high-oleate sunflower oil (SVO Specialty Products, Inc., Eastlake, OH) to produce oil for cheesemaking. Interesterification was carried out in several batches for each oil type. Reagent amounts were 2,212 g high-oleate sunflower oil, 288 g SCTG, and 19.6 mL 5.4 *M* sodium methoxide in methanol (Fluka Chemical Co, Ronkonkoma, NY). These oil ratios simulated the proportion of short-chain fatty acids to total fat in milk fat.

Methanol was evaporated from the catalyst under vacuum in a 3-L flask. When the catalyst was dry, the high-oleate sunflower oil and SCTG were immediately added. The flask headspace was flushed with nitrogen gas, and fitted with a Tru-bore glass stirring rod and teflon paddle. The reaction mixture was heated to 65°C with vigorous stirring for 22 h. The sodium methoxide catalyst was neutralized with 5% aqueous acetic acid, and the oil was washed with distilled water and dried over anhydrous sodium sulfate. An Acid Value Test was conducted, and

values less than or equal to 2.0 mg KOH/g sample were accepted. Batches were pooled for deodorization.

Deodorization of Oils

Interesterified oils were deodorized in 1-L batches to remove residual solvents, fatty acid methyl esters, partial glycerides, short-chain triglycerides, and off-flavors. The deodorization apparatus is shown in Figure 2. Each batch of oil was deodorized at 240°C for one hour, and cooled to room temperature for tasting. If the oil did not taste similar to fresh refined, bleached, and deodorized high-oleate sunflower oil, it was subjected to further deodorization. Not more than two hours of deodorization was needed for any oil. To determine final acceptability, oils were prepared and tasted as emulsions following the method of Stone and Hammond (1983). Batches of each oil type were pooled and tasted again. Gas chromatography analysis was performed to determine the final fatty acid profile of each oil. For each 1-L batch of oil that was deodorized, roughly 60 mL of dry ice/acetone trap contents were recovered.

Preparation of Cultures Used For Cheesemaking

L.actobacillus bulgaricus AR2, *Streptococcus thermophilus* AC2, and *Propionibacterium shermanii* P19 were obtained from the culture collection of the Department of Food Science and Human Nutrition at Iowa State University. *L. bulgaricus* AR2 and *S. thermophilus* AC2 were grown in 94.8 g/L reconstituted Carnation natural nonfat dry milk (Carnation Co., Los Angeles, CA) at incubation temperatures of 40°C and 37°C, respectively. *P. shermanii* P19 was grown in sodium lactate broth (Odazali, 1992) at 35°C. Eighteen-hour cultures of each

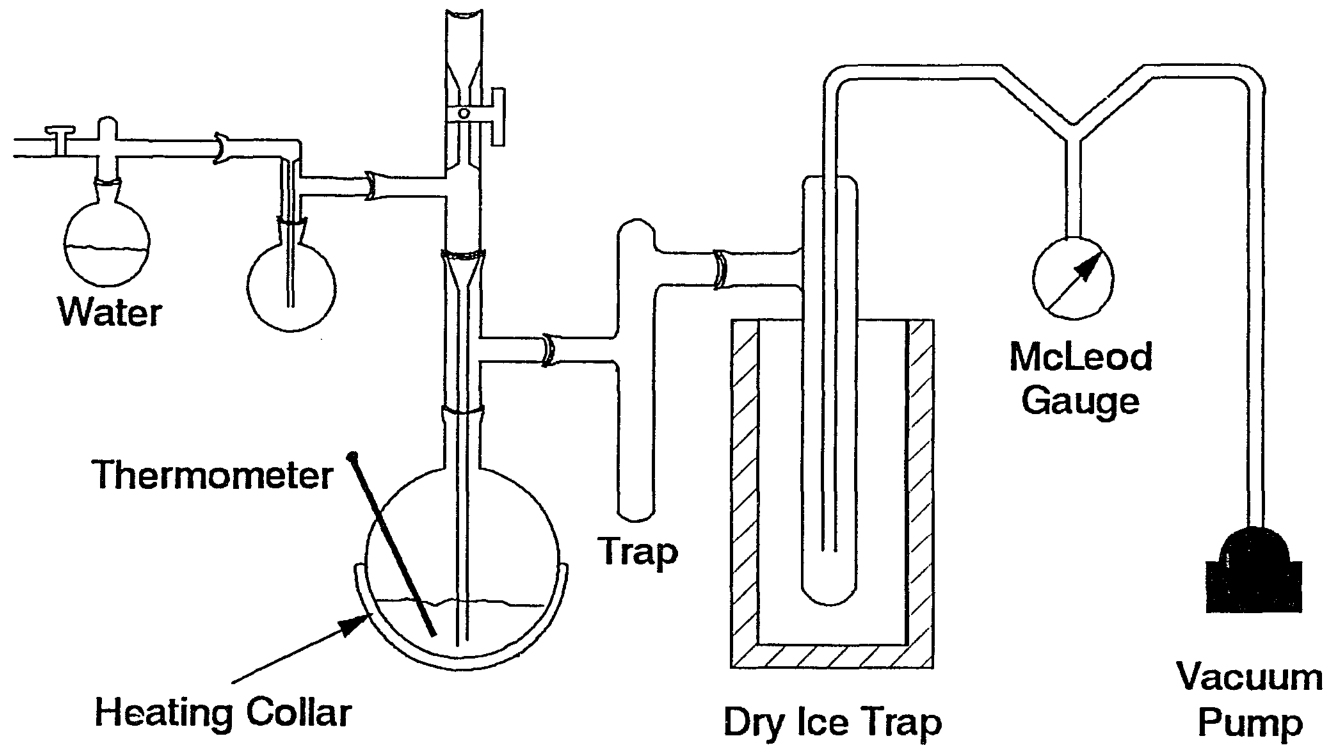

Figure 2. Schematic diagram of oil deodorization apparatus

organism grown in the appropriate medium were frozen in 1.0-mL batches containing equal volumes glycerol and culture broth, and stored at -80°C until needed.

When a culture was needed for cheesemaking, it was thawed, inoculated at 1% (v/v) into the appropriate medium, and incubated for approximately 36 hrs. *S. thermophilus* AC2 was incubated at 40°C, *P. shermanii* P19 at 32°C, and *L. bulgaricus* AR2 at 37°C. Two additional transfers at 1% (v/v) of 18-h cultures were made to obtain active cultures.

All 18-h cultures contained $2.0x10^9$ - $3.3x10^9$ CFU/mL as determined using Standard Plate Count Agar (Difco, Detroit, MI) for *S. thermophilus*,AC2, sodium lactate agar for *P. shermanii* P19, and MRS Lactobacilli agar for *L. bulgaricus* AR2. Absorbance at 475 nm was determined for 18-h cultures of *P. shermanii* by means of an Hitachi U-2000 Spectrophotometer (Hitachi, NY, NY). One milliliter samples of culture were diluted in 9.0 mL water before absorbance readings were taken. This was done in triplicate for two experiments. Values ranged from A_{475} = 0.380 to A_{475} = 0.420 for the two experiments. It was not possible to take absorbance readings for the *S. thermophilus* and *L. bulgaricus* cultures, because of interference by milk proteins.

A commercial mesophilic homofermentative starter culture R-603 was obtained from Chr. Hansen's Laboratory, Inc. (Milwaukee, WI). This starter culture was stored at -80°C until needed. When this culture was needed for cheesemaking, it was added directly to the milk in its frozen pelletized form without preincubation.

<u>Cheesemaking</u>

Skim milk, processed by Anderson Erickson, Des Moines, IA, was purchased from a local grocery store. When control cheeses were made from natural milk emulsion, raw milk was obtained from the Iowa State University Dairy Teaching and

Research Center Holstein herd, Ames, IA. The raw milk was pasteurized at 71.5°C using an Agric Machinery Model 10760 plate heat exchanger-type pasteurizer (Agric Machinery Corp., Madison, NJ).

Iowa Style Swiss Cheese was made following the procedure of Reinbold (1972). Steps for cheesemaking are outlined in Table 1

Cheeses were made from the following ingredients: natural milk emulsion (CONTROL1); homogenized natural milk emulsion (HOMOGCONT); recombined skim milk and milk fat (FAT/SKIM); recombined skim milk, milk fat and buttermilk solids (BUTTERMILK); recombined skim milk, milk fat and gum acacia (GUM ACACIA). Buttermilk solids were obtained from Farmers' Cooperative Creamery, McMinville, OR. The amount of buttermilk solids used in each batch of cheese was equal to the amount of buttermilk solids by weight that would have been naturally associated with the added fat. The type of gum acacia used was TIC Pretested® Arabic FT Powder obtained from TIC Gums (Belcamp, MD). This type was chosen for its high quality, and its desirable organoleptic, physical and chemical, and bacteriological profiles. It was used at a level of 3% of the fat by weight. Milk fat was prepared as described previously.

A second set of cheeses was made from the following ingredients: natural milk emulsion (CONTROL2); recombined skim milk, high-oleate sunflower oil and gum acacia (SUN); recombined skim milk, natural milk fat SCFA interesterified with high oleate sunflower oil and gum acacia (BUTTER/SUN); recombined skim milk, synthetic SCFA interesterified with high-oleate sunflower oil, and gum acacia (SIGMA/SUN).

In order to incorporate the added oils into the skim milk, it was necessary to homogenize the ingredients. A Gaulin two-stage homogenizer, Model 18M-8TA (AVI Gaulin Corp., Wilmington, DE), was used. The first stage set at 140 kg/cm^2, and the second stage at 50 kg/cm^2. One half of the total skim milk used for each batch was heated in the cheese vat to 32°C while stirring. Oil and buttermilk solids or gum acacia, where indicated, were added and stirred for 15 minutes. Then, this

Table 1. Procedure for manufacture of "Iowa-Style" Swiss cheese

Operation	Time	Temperature	Remarks
Fill vat [a]		32°C	approx.120 kg milk
Add starter [b]		32°C	
Ripening	30 min.	32°C	
Renneting [c]	30 min.	32°C	
Cutting	10 min.	32°C	0.64 cm knives
Foreworking	10 min.	32°C	
Whey Removal		32°C	
Cooking		41°C	
Stir-out	2 h from start of cut	41°C	
Vat Pressing	30 min. under whey 30 min. without whey		25 kg weight
Hoop Pressing	approx. 18 h	22-25°C	9 kg Wilson hoop
Brine Salting	2 days	41°C	
Drying	several hours	41°C	
Packaging		22-25°C	Vacuum packaging
Cold Room	10 days	7°C	
Warm Room	3 weeks	25°C	
Finished Cooler	3 months from start of process	4°C	

[a] Adjusted to 2.8% milk fat

[b] Inoculation ratio used: for 120 kg milk, use 15.6 g lactic culture, 4.8 g *L. bulgaricus* AR2, 284.5 g *S. thermophilus*, 17.3 g *P. shermanii.*

[c] Calf rennet (chymosin), 90 ml/455 kg.

mixture was pumped continuously to the homogenized using a Cole Parmer Masterflex peristaltic pump (Cole Parmer, Niles, IL) fitted with a #7018-52 pump head and #6019-18 Masterflex Tygon food grade tubing. Flow rate was approximately 1 L/min. Ingredients entering the homogenizer feed tank were stirred continuously, and homogenized product was collected in a sanitized milk can. This product was returned to the cheese vat when homogenization was complete, and the remainder of the milk was added. After heating to 32°C while stirring, the cheese was made.

Packaging of Cheese

After the brining step, cheeses were dried and packaged using a Koch vacuum packaging machine (Koch Supplies, Inc., Kansas City, MO). Packaging material was Curlon® Grade 861 polyethylene, with a film thickness of 2.7 mil (Curwood, Oshkosh, WI). Following three months of aging, each block of cheese was cut into 20 portions weighing approximately 1-lb each by using a pneumatic cutting machine equipped with stainless steel cutting wire. The 1-lb portions were repackaged as before and stored at -20°C until needed.

Cheese Sample Preparation

Cheese samples for various chemical tests were taken from the center portion of each block. First, a one-pound portion of each cheese was thawed overnight at 4°C. Then, the one-pound block of cheese was grated using a kitchen grater, and the gratings were mixed before a sample was taken.

Free Fatty Acid Analysis

Determination of the free fatty acid content in cheese followed the method of de Jong and Badings (1990), with several minor modifications. Duplicate 1-g samples of cheese were ground separately with 3.0 g anhydrous sodium sulfate using a mortar and pestle. Each sample was transferred to a vial, to which 0.3 mL 2.5*M* sulfuric acid was added. In order to check the recovery of individual free fatty acids by the extraction step, 1 µl of an internal standard composed of 0.5 mg/mL each C5:0, C7:0, C13:0 and C17:0 was added to the sample. After a brief centrifugation, the mixture was extracted three times with 3 mL diethyl ether:heptane 1:1, v/v, and the extracts were pooled.

Solid phase extraction columns containing 500 mg aminopropylsilyl phase (2.8 mL volume) packed between stainless steel frits (Alltech Associates, Inc., Deerfield, IL) were used to separate the different lipid classes in the samples. The aminopropyl column was first conditioned with 10 mL heptane, and the sample was applied to the column. The neutral lipids were extracted with 4 mL chloroform:2-propanol, 2:1 v/v. The free fatty acids were extracted with 4 mL of 2% formic acid in diethyl ether. To the free fatty acid extract, 1 mL of an injection standard containing 0.5 mg/mL C11:0 was added. For GC analysis, 1 µL of this sample was injected using equipment described previously. The GC oven was held at 40°C for 2 min, programmed at a rate of 10°C/min until a temperature of 200°C was reached, and held at 200°C for 10 min. Both the injection port and the flame ionization detector (FID) were set at 220°C. Recovery factors for the extraction step are given in Table 2.

In order to quantify individual free fatty acids in the cheese samples, correction factors for the detector response were calculated using the approach of Ackman and Sipos (1964). Correction factors were calculated relative to the individual responses of the internal standards C5:0, C7:0, C13:0 and C17:0. de Jong and Badings (1990) found that long-chain free fatty acids of chain length

greater than or equal to C16:0 gave lower FID responses than did C12:0, and I also found this to be true. Thus, the free fatty acids were divided into chain-length groups. Each internal standard component within the chain-length groups was given the value of 1.0, and correction factors were determined relative to this standard compound. Correction factors are given in Table 2.

Minor Short-chain Free Fatty Acid Analysis

Samples were prepared as described above. Since the minor SCFAs are present in small quantities in milk fat, it was necessary to concentrate the samples. To do this, 1 mL of each sample was evaporated at room temperature to approximately 50 μL. Then, 50 μL of C13:0 internal standard, 5 mg/mL, was added to the sample. Gas chromatography analysis was performed as described above.

Fat Determination

Fat content of cheese samples was determined by the AOAC Roese-Gottleib Method, 905.02 (1990). One-gram cheese samples, 9 mL water, and 2 mL NH_4OH were used.

Moisture Determination

Moisture content of cheese samples was determined by the AOAC Method I, 926.08 (1990).

Table 2. FID [a] response factors, extraction recoveries, [b] and isolation recoveries [c] for free fatty acid analysis in whole cheese

Fatty Acid	Isolation	Recovery	FID
C2:0	0.61	0.85	2.3499
C3:0	0.90	0.90	1.4491
C4:0	0.96	0.93	1.1503
C6:0	1.00	0.89	1.0711
C8:0	0.99	0.93	0.9494
C10:0	1.00	0.89	1.0716
C11:0	1.00	1.00	1.0429
C12:0	1.05	0.86	1.0195
C14:0	0.96	0.77	0.9835
C16:0	0.97	0.76	1.0013
C18:0	0.93	0.94	0.9900
C18:1	0.99	1.00	0.9829

[a] FID = Flame ionization detector.

[b] Extraction = fat extraction from whole cheese.

[c] Isolation = recovery from aminopropylsilyl cartridge.

pH Determination

One-gram samples of grated cheese were kept in 10 mL distilled water for several minutes, and then stirred thoroughly. The pH was measured with a standard combination calomel-glass electrode at 25°C. The electrode was cleaned thoroughly with 1*M* HCl and 1*M* NaOH after each measurement to avoid drifting due to protein accumulation on the probe. After each cleaning, the pH meter was recalibrated with pH 4.00 and pH 7.00 buffers.

Proteolysis Determination

Proteolysis was determined using the method of Vangtal and Hammond (1986). Glycine standards were prepared by the method of Samples et al. (1984), using glycine concentrations ranging from 0.005 *mM* to 0.1 *mM*. Absorbance was determined with an Hitachi U-2000 Spectrophotometer (Hitachi Instruments, Japan) equipped with a deuterium lamp.

Sensory Analysis of Cheese

Sensory evaluation for taste and flavor of the cheeses was performed using a 12-member trained descriptive sensory panel for the first group of cheeses, and 15 individuals for the second group of cheeses. The four primary taste attributes, sweet, salty, acid, and bitter were evaluated. Flavor concepts were developed by the panelists, and consisted of terms that described particular known aroma sensations. Individuals volunteering to participate on the panels were Iowa State University faculty, staff, and students. All panelists were regular cheese eaters who had tasted Swiss cheese before. Cheeses included in the first group were

CONTROL1, HOMOGCONT, FAT/SKIM, BUTTERMILK, and GUM ACACIA. Cheeses included in the second group were CONTROL2, SIGMA/SUN, BUTTER/SUN, and SUN.

Training of the panelists for each sensory evaluation was conducted over a period of four consecutive days, in sessions of one-half hour each. During the training sessions, panelists tasted several domestic and imported Swiss cheeses. These cheeses were chosen to represent a wide range of quality and to contain varying intensities of different flavor notes. Panelists developed terminology to describe particular flavor attributes of the cheeses. After discussion and preliminary evaluation, panelists were trained to identify and evaluate different intensities of four flavor concepts: "buttery," a diacetyl-like flavor; "burned /caramelized," a burned sugar flavor; "volatile," the sensation of aromas filling the oral cavity; and "typical Swiss cheese," a complex nutty-type flavor normally associated with Swiss cheese. The four tastes, sweet, salty, acid, and bitter, were also evaluated. For the second group of cheeses, it was also necessary for the panelists to evaluate the cheeses for a flavor concept known as "lipolyzed," a flavor defect in Swiss cheese characterized by excessive release of free SCFAsfrom the triglycerides. Panelists were trained to ignore any textural differences that existed between the samples.

Cheese samples were cut from the center portion of each block into 2-cm cubes, and served at room temperature. Because of the flavor complexity of the product, only four samples were evaluated each day. Panelists performed replicate evaluations of coded, randomly presented samples in individual booths illuminated with red fluorescent lights. Evaluation took place during morning sessions, and panelists were first asked to chew a sample of commercial cheese in order to accustom their palates to the taste of cheese Then, evaluation of samples began.

Panelists were instructed to chew each sample thoroughly and allow the volatile flavor compounds to fill the oral cavity. Then, each attribute was evaluated in the order in which it was presented on the sensory evaluation form (Figure 3). Fifteen-centimeter line scales with intensity anchors printed under both ends of each

Sensory Analysis

Name:______________________________ Date:_________

Directions: Place a line (slash) perpendicular to the line scale at the point which best describes your evaluation of each attribute. Label each mark with the sample code number.

Sweet

Low High

Salty

Low High

Acid

Low High

Bitter

Low High

Buttery

Undetectable Strong

Volatile

Weak Strong

Typical Swiss Cheese

Weak Strong

Burned/Caramelized

Undetectable Strong

Figure 3. Sample of a sensory evaluation score sheet

line were used. The intensity of each attribute was indicated by placing a slash perpendicular to the corresponding line scale according to the panelist's perception. Between samples panelists were asked to rinse their mouths with tap water at room temperature.

Scores generated by individual panelists for each sample were determined by measuring in centimeters from the left side of each line scale. These raw data were then analyzed statistically.

Textural Evaluation of Cheeses

A 6-member trained sensory evaluation panel was used for evaluation of the texture of the cheeses. Panelists were trained to evaluate "hardness" and "crumbliness" of the cheese using previously frozen and thawed commercial domestic Swiss cheese. "Hardness" was defined as the degree of firmness experienced during the first bite of the cheese using the molars. "Crumbliness" was defined as the degree of crumbling during the first ten chews. During evaluation sessions, a calibration sample was given to establish the midway point of the hardness scale, and the three-quarter point of the crumbliness scale. Panelists were trained to ignore taste, flavor, and appearance characteristics of the cheeses.

Cheeses were evaluated using two 15-centimeter line scales (Figure 4), with intensity anchors printed under both ends of each line. Intensity anchors for hardness, from left to right, were "soft" and "firm," respectively. Intensity anchors for fracturability, from left to right, were "crumbly" and "smooth," respectively. Panelists evaluated nine samples each day. Cheese samples were prepared as described previously. Panelists performed repeated measure evaluations of coded, randomly presented samples under incandescent light at a large conference table. Scores generated by individual panelists for each sample were determined by measuring in

TEXTURE EVALUATION

Date____________________ Name___________________

Instructions: Chew each sample of cheese approximately ten times. Wash your mouth out between samples; you are not required to swallow the samples. For evaluation, place a line (slash) perpendicular to the line scale at the point which best describes your evaluation of each attribute. Label each mark with the sample code number. Ignore flavor and taste differences; concentrate only on texture characteristics. A calibration sample is provided to establish 3/4 point on the "crumbliness" scale, and 1/2 point on the "hardness"scale.

Hardness

____

Soft Firm

Crumbliness

____

Crumbly Smooth

Figure 4. Sample of a texture evaluation score sheet

centimeters from the left side of each line scale. These raw data were then analyzed statistically.

Statistical Analysis

A SAS program, Proprietary Software Release 6.06, was used for statistical analyses (SAS Institute Inc., 1989). General Linear Models Procedure (GLM) was used to analyze sensory panel data, chemical data, and physical data. Pearson's Correlation coefficients and least significant difference (LSD) means were also determined. Factor analyses were performed using PROC FACTOR. Multiple regression analysis was also performed for some data.

RESULTS AND DISCUSSION

Modification of the Method For Determination of α-Dicarbonyls

The sample preparation and α-dicarbonyl detection methods used in this work have been modified from the methods of Bednarski et al. (1989). The solvent used in sample preparation was changed from chloroform to diethyl ether in order to reduce solvent evaporation time. Confirmation of the present sample preparation method was performed in two ways. For confirmation of the extraction/evaporation step, known quantities of quinoxaline, methylquinoxaline, and dimethylquinoxaline were added to Lactobacilli MRS broth, and extracted three times with diethyl ether. Samples were evaporated and prepared as described previously, and injected onto the HPLC column. Results were compared with standard curves for each compound, and it was determined that the extraction/evaporation method gave 100% recovery.

The derivatization step was tested by adding known quantities of glyoxal, methylglyoxal, and dimethylglyoxal to Lactobacilli MRS broth. Derivatization yielded quinoxaline, methylquinoxaline, and dimethylquinoxaline, as described previously. Samples were quantified by HPLC. Based upon standard curves for each derivative, it was determined that this step gave 100% recovery of the added dicarbonyls. A control sample in which no dicarbonyls were added to the Lactobacilli MRS medium showed no detectable amounts of dicarbonyls when subjected to this method of analysis.

Because of the recent commercial availability of HPLC-grade quinoxaline standards, the use of skatole as an internal standard was abandoned. Standard curves prepared daily gave consistent results. A wavelength scan was conducted to determine the optimal wavelength setting for the detection of quinoxalines. It was

discovered that at 254 nm the quinoxaline peak was not being measured optimally. Therefore, the UV detector wavelength used in this study was set at 315 nm instead of 254 nm to give optimal performance. This finding is confirmed by Tricomi et al. (1994).

The HPLC conditions were altered to give improved separation of quinoxaline derivatives, and thus maximal quantitation of peaks. The HPLC solvent ratios used by Bednarski et al. (1989) gave incomplete separation of quinoxaline and an unidentified peak. This resulted in inconsistent data for glyoxal. To identify the unknown peak, microbial metabolites that could be present in the samples were considered. Dihydroxyacetone phosphate is a component in the Embden-Meyerhof pathway (Stryer, 1988); when the phosphate group is removed, dihydroxyacetone, an α-hydroxycarbonyl, is left. Thus, dihydroxyacetone could conceivably be present in varying levels in all samples during the lifetime of the cells.

To test for the presence of dihydroxyacetone in the samples, known quantities of dihydroxyacetone were added to aliquots taken from culture tubes after incubation and just prior to sample preparation. The unknown peak areas from these aliquots were compared to the peak areas from aliquots not containing added dihydroxyacetone. Peaks from the treated samples were much larger than peaks from untreated samples. To further confirm the identity of the unknown peak, known amounts of dihydroxyacetone were added to unincubated Lactobacilli MRS broth, and samples were prepared and analyzed as before. This gave a peak with the same retention time as the unknown peak, while control samples containing only Lactobacilli MRS broth showed no peak. It was concluded that the unknown peak was dihydroxyacetone.

Factors Affecting Cell Viability and Dicarbonyl Production By *Lactobacillus bulgaricus* AR2

Cell Viability of *Lactobacillus bulgaricus* AR2 Following Heat-Shock Treatment

Control of temperature is a very important aspect of Swiss cheese production. As can be seen from Table 1, a 2-h cooking/stir-out step at 41°C is an integral part of processing. The purpose of this step is to remove moisture from the curd so that a firm, elastic, leathery body can form (Reinbold, 1972). Cooking is also a critical step for the control of acid production and improved whey drainage during pressing (Reinbold, 1972). However, because cultures used in Swiss cheese production are sensitive to temperatures above 49°C, it is important to maintain strict temperature control throughout the cooking process.

Results of this study indicate that, after 24 h and 48 h of post-treatment incubation, there was no significant difference in viability of *L. bulgaricus* AR2 among samples that were heat-shocked at 42°C or 50°C, and control samples that received no heat-shock treatment. After 72 h of post-treatment incubation, both heat-shocked treatments yielded significantly fewer viable cells than did control samples. Seventy-two hour samples that were not heat-shocked had cell viability that was not significantly different from all 24 h and 48 h samples. Cell counts for all samples were similar to those reported by Biede et al. (1979). These results (Table 3) indicate that some cell viability of heat-shocked samples is lost after three days of incubation.

Table 3. Viability of *Lactobacillus bulgaricus* AR2 following heat-shock treatment at 42°C or 50°C

Sample[c]	42°C	50°C	No heat-shock
0 h	9.49 ± 0.04 [A]	9.49 ± 0.04 [A]	9.49 ± 0.04 [A]
24 h	9.63 ± 0.17 [A]	9.47 ± 0.05 [A]	9.52 ± 0.16 [A]
48 h	9.49 ± 0.07 [A]	9.50 ± 0.17 [A]	9.51 ± 0.08 [A]
72 h	8.49 ± 0.08 [B]	8.27 ± 0.16 [B]	9.51 ± 0.11 [A]

[a] Values with different superscripts within the same column are significantly different at $p<0.05$. Values represent the average of three replicate measures from two experiments.

[b] Log_{10} viable cell count, Lactobacilli MRS agar. Limit of detection is 1.0×10^2 CFU/mL.

[c] Samples incubated at 37°C.

Cell Viability and Dicarbonyl Production by *Lactobacillus bulgaricus* AR2 in Sodium Oleate-Containing Medium

Anders and Jago (1970a) speculated that oleic acid inhibits the growth of *Streptococcus cremoris* C13 in Cheddar cheese during the early stages of ripening. Czulak et al. (1979) found similar results in Cheddar cheeses made with milk whose fat was rich in linoleic acid. In another study, Anders and Jago (1970b) found that the dehydrogenase systems coupled to the metabolism of pyruvate in whole-cell suspensions of *S. cremoris* C13 and HP in various buffers were inhibited over a pH range of 4.5 to 6.5. They postulated that the inhibition of cell growth and enzyme system function could significantly inhibit the production of important flavor compounds such as diacetyl in cultured dairy products.

Using Lactobacilli MRS broth at pH 6.2, I found little evidence of loss of cell viability in the presence of 5.0 *mM* oleic acid. At 24 and 48 h, there was no significant difference in the cell viability of *Lactobacillus bulgaricus* AR2 in samples grown in the presence or absence of 5.0 *mM* oleic acid. However, at 72 h, cell viability in the presence of 5.0 *mM* oleic acid was significantly lower than cell viability in the absence of 5.0 *mM* oleic acid. At 96 h, there was little viability of cells in both samples. Results are shown in Table 4. The results of this experiment support the conclusion of Law et al. (1972) that long-chain fatty acids do not inhibit the growth of starter cultures used in cheesemaking.

Findings of these experiments do not corroborate the conclusions of Anders and Jago (1907b) that cell growth and dimethylglyoxal (diacetyl) production in Group N streptococci is inhibited in the presence of oleic acid. In this study, dimethylglyoxal (diacetyl) production by *L. bulgaricus* AR2 in the presence or absence of 5.0 *mM* oleic acid was not significantly different across treatments at any measurement interval. Dimethylglyoxal levels were low compared to other dicarbonyl levels, ranging from 0.00 μg/mL to 1.11 μg/mL.

Table 4. Viability of *Lactobacillus bulgaricus AR2* in 5.0 *mM* sodium oleate Lactobacilli MRS broth [a,b]

Sample[c]	0.0 mM NaOle. [d]	5.0 mM NaOle. [d]
0 h	9.49 $\pm$ 0.04 [A]	9.49 $\pm$ 0.04 [A]
24 h	9.34 $\pm$ 0.05 [B]	9.37 $\pm$ 0.08 [A,B]
48 h	9.35 $\pm$ 0.11 [A,B]	9.25 $\pm$ 0.13 [B]
72 h	9.67 $\pm$ 0.15 [A]	7.33 $\pm$ 0.48 [C]
96 h	undetected [D]	undetected [D]

[a] Values with different superscripts within the same column are significantly different at $p<0.05$. Values represent the average of three replicate measures from two experiments.

[b] Log_{10} viable cell count, Lactobacilli MRS agar with or without 5.0 *mM* sodium oleate. Limit of detection is 1.0×10^2 CFU/mL.

[c] Samples incubated at 37°C.

[d] NaOle. = sodium oleate.

Results for all dicarbonyl determinations performed in this experiment are shown in Table 5. Because Anders and Jago did not test for the production of glyoxal and methylglyoxal, no comparison between studies can be made. In this study, at 24, 48, and 72 h of incubation in the presence or absence of 5.0 *mM* oleic acid, no glyoxal was detected in either treatment. Glyoxal production by cells grown in the presence or absence of 5.0 *mM* oleic acid was not significantly different between samples at 96 h. Ninety-six hour glyoxal levels (approximately 30.00 μg/mL) were the highest of all dicarbonyl levels measured.

Methylglyoxal production by *L. bulgaricus* AR2 ranged from 0.00 μg/mL to 12.82 μg/mL. Production of methylglyoxal at 24 h was significantly higher in samples grown in the absence of oleic acid. At 48 h, methylglyoxal levels in both treatments were significantly lower than in 24-h samples, with the 48-h control (no oleic acid) samples being significantly lower than 48-h samples with added oleic acid. Seventy-two hour samples had the highest methylglyoxal levels measured. They were not significantly different between treatments, nor were they different from 24-h samples grown in the absence of oleic acid. At 96 h, methylglyoxal was not detected in any samples; this was significantly lower than all other measurement intervals up to 72 h for both treatments. In general, both treatments followed the same increase and decrease trends in methylglyoxal production. Methylglyoxal was not detected at 96 h, yet this measurement interval yielded the first evidence of glyoxal production. These results suggest that methylglyoxal could be a metabolic precursor to glyoxal production.

Several investigators have demonstrated the importance of microbially produced α-dicarbonyls such as glyoxal, methylglyoxal, and diacetyl in the generation of characteristic Swiss cheese flavors (Reps et al., 1987; Griffith and Hammond, 1989; Bednarski et al., 1989). Griffith and Hammond showed that Swiss cheese flavor can be formed *in vitro* by the reaction of α-dicarbonyls with amino acids, especially valine, leucine, isoleucine, phenylalanine, lysine, methionine, and

Table 5. Dicarbonyl production by *Lactobacillus bulgaricus* AR2 grown in 5.0 *mM* sodium oleate MRS broth [a]

Sample [b,c]	Glyoxal [d] (ug/mL)	Methylglyoxal [d] (ug/mL)	Diacetyl [d] (ug/mL)
5.0 mM, 24 h	not detected [B]	11.53 ± 0.26 [B]	0.07 ± 0.12 [B]
5.0 mM, 48 h	not detected [B]	10.51 ± 0.39 [C]	1.11 ± 0.38 [A]
5.0 mM, 72 h	not detected [B]	12.71 ± 0.03 [A]	0.20 ± 0.16 [B]
5.0 mM, 96 h	30.36 ± 4.27 [A]	not detected [E]	not detected [B]
0.0 mM, 24 h	not detected [B]	12.71 ± 0.40 [A]	0.09 ± 0.16 [B]
0.0 mM, 48 h	not detected [B]	9.78 ± 0.26 [D]	0.71 ± 1.24 [A,B]
0.0 mM, 72 h	not detected [B]	12.82 ± 0.82 [A]	0.41 ± 0.53 [A,B]
0.0 mM, 96 h	30.65 ± 2.51 [A]	not detected [E]	not detected [B]

[a] Values with different superscripts within the same column are significantly different at $p<0.05$.

[b] 0.0 mM = no sodium oleate added to Lactobacilli MRS medium.

[c] 5.0 mM = 5.0 mM sodium oleate added to Lactobacilli MRS medium.

[d] Limit of detection = 0.05 ug/mL.

cysteine. Flavorful products from α-dicarbonyl/amino acid reactions are produced via the well-known Strecker degradation (Kowalewska et al., 1985; Griffith and Hammond, 1989). Methylglyoxal can polymerize to form 2,5-dimethyl-4-hydroxy-3(2H)-furanone, which gives a caramel or burned sugar flavor (Kowalewska et al., 1985; Griffith, 1988).

In the early 1900s, researchers speculated that methylglyoxal had a role in glycolysis (Dakin and Dudley, 1913). They proposed that methylglyoxal was formed from dihydroxyacetone phosphate, and then converted to D-lactate, which could then be converted to pyruvate. The existence of an enzyme system for the conversion of methylglyoxal to D-lactate is now known to contain two enzymes, namely glyoxalase I (EC 4.4.1.5: *S*-lactoylglutathione methylglyoxal lyase) and glyoxalase II (EC 3.1.2.6: hydroxyacylglutathione hydrolase) (Racker, 1951). In the 1970s, methylglyoxal synthase (EC 4.2.99.11) was shown to be the enzyme responsible for the formation of methylglyoxal from dihydroxyacetone phosphate. These enzymes are present in a variety of bacteria (Cooper, 1984) and could provide a bypass of the Embden-Meyerhof pathway for the formation of pyruvate. Methylglyoxal can also be produced microbially by other means (Taylor et al., 1980). However, if the methylglyoxal bypass of the Embden-Meyerhof pathway were to occur in dairy cultures, it would follow that inhibition of dehydrogenase systems coupled to the metabolism of pyruvate would not necessarily inhibit cell growth, as postulated by Anders and Jago (1970b).

The glyoxal levels reported in these studies are much lower than those reported by Bednarski et al. (1989) for *L. bulgaricus* AR2 grown in Lactobacilli MRS medium. Methylglyoxal and diacetyl levels reported by Bednarski et al. (1989) are within the same ranges as reported here. It is possible, since interference by dihydroxyacetone was not considered in earlier studies, that the high glyoxal levels reported by Bednarski et al. (1989) unknowingly represented glyoxal + dihydroxyacetone.

Preparation of Milk Fat Fatty Acid Methyl Esters and Free Fatty Acids

Because relatively large quantities of C4:0-C12:0 free fatty acids isolated from natural milk fat were needed for triglyceride synthesis and emulsion preparation, several batches of milk fat fatty acid methyl esters (FAME) were prepared using the acidic methanol method. After the preparation of each batch, the product was checked for residual mono-, di- and triglycerides using conventional TLC methods. Presence of these components indicates an incomplete reaction. When this was the case, the sample was subjected to a second reaction using excess methanol and freshly prepared sodium methoxide. It is possible that some batches did not go to completion because of insufficient reaction time, or because of the very large reaction volumes (>3000 mL) used upon several occasions. Reaction volumes of less than 3000 mL are recommended for each batch.

Once all batches of FAME had been prepared, they were pooled, and GC analysis was performed. This gave quantitative values for the fatty acid components of the pooled sample, as shown in Table 6, with C4:0-C12:0 FAME representing approximately 12 percent of the total FAMEs. The wide-bore Nukol® column proved to be an excellent choice for separation of the different FAMEs in less than 30 min. FAME analyses for the milk fat gave results comparable to published data, as shown in Table 7. As compared to these published data, it is evident that values obtained in this study for C10:0 and C12:0 are low, while C6:0 is high. Values for C4:0 and C8:0 are close to the published values.

Distillation of the FAMEs to yield a C4:0-C12:0 fraction resulted in some losses of C4:0, despite the use of a dry ice/acetone trap. However, these losses were small, and are considered to be mechanical losses. Control of the vacuum leak (see Figure 1) and the Cartesian manostat were crucial in controlling the vacuum of the system. Several distillations were required to process all of the FAMEs, with each batch containing approximately 1000 mL of starting material. After each distillation, the pot liquor was checked for residual C4:0-C12:0 using the

Table 6. Molar ratios of C4:0-C12:0 FAME isolated from milk fat

FAME [a]	Mole %
C4:0	47.0
C6:0	29.8
C8:0	13.5
C10:0	8.5
C12:0	1.2

[a] FAME = fatty acid methyl ester.

Table 7. Molar ratios of C4:0-C12:0 milk fat as methyl esters calculated from Jensen, 1992 [a]

FAME [b]	Mole %
C4:0	41.4
C6:0	17.4
C8:0	8.3
C10:0	16.3
C12:0	16.6

[a] Table adapted from this publication.
[b] FAME = fatty acid methyl ester.

GC method. If any of these FAMEs were still left in the pot, the FAMEs were distilled again. The distillation products from each batch were pooled.

Cold saponification was used to liberate the free fatty acids from the methyl esters (Markley, 1964). The reaction proceeds rapidly and exothermally following the addition of the ethanol catalyst. The product was checked for residual FAME by conventional TLC and GC analysis using the Nukol® column. Free fatty acids were recovered after acidification of the soaps. For this step, the reaction flask was immersed in an ice bath, and a water-cooled condensing column was quickly placed on the flask after addition of the acid in order to prevent volatiles from escaping. Because of the high salt concentration in the aqueous phase following acidification, losses of C4:0 into the aqueous phase were not of any consequence once the solution was cooled.

Natural Milk Fat and Synthetic Milk Fat Fatty Acid Emulsions

Emulsions of natural and synthetic fatty acids were tasted in order to determine if flavor differences existed. While the natural fatty acid emulsion contained all major normal even-chain as well as minor odd, unsaturated and branched chain fatty acids from C4:0 to C12:0 that are normally found in milk fat, the synthetic mixture contained only the major normal even-chain fatty acids. The judges found that the flavor of the natural emulsion was much more intense and cheese-like than the synthetic emulsion. This confirms the report of Ha and Lindsay (1988) that the minor branched and unsaturated SCFAs of milk fat are important in cheese flavors. Because many of these fatty acids are not readily available from commercial suppliers, cheeses were made in which either synthetic mixtures of the major short-chain fatty acids of milk fat or the natural short-chain fatty acids from milk fat were interesterified into high-oleate sunflower oil.

Short-chain Triglyceride Preparation and Interesterification Into High-oleate Sunflower Oil

Earlier work by Johnson (1991) showed that, as a first step in the interesterification of vegetable oils andSCFAs, short-chain triglycerides must be made. Other researchers have synthesized structured triglycerides for food use by interesterifying mono-species short-chain triglycerides such as tributyrin or triacetin with long-chain triglycerides such as soybean oil or cottonseed oil (Klemann et al., 1994). The approach taken in this study, however, is different in that the short-chain triglycerides used in the final oil synthesis were not mono-species; they were random combinations of C4:0-C12:0 fatty acids. For our purposes, the distribution of the various fatty acids on the glycerol backbone was assumed to be random, based on evidence provided by the exhaustive studies of Klemann et al. (1994).

The triglyceride synthesis method of Quinn (1967) is a reliable and easy method. The reaction endpoint can be easily estimated, as no more water of reaction will be produced. Each batch was tested for residual mono- and di-glycerides using conventional TLC. If any were detected, the reaction was continued. Following adequate neutralization of the acid catalyst, the batches were pooled. Care must be taken to fully neutralize the acid catalyst, otherwise, burning may occur in subsequent processing of the triglycerides.

As noted by Johnson (1991), the short-chain triglycerides produced in this manner were quite bitter tasting. Thus, they could not simply be mixed with the high-oleate sunflower oil, or a bitter taste would predominate. Instead, they had to be randomized as completely as possible with the high-oleate sunflower oil through an interesterification reaction. Following interesterification, each batch was analyzed for gross composition using conventional TLC. These results showed that some triglycerides containing two or three short-chain fatty acid groups remained. These triglycerides (SSS, SLS, or SSL) were distinguished from triglycerides containing at least two long-chain fatty acids (LLL, LSL, or SLL) on the TLC plates,

since they did not migrate as far from the origin as did the long-chain triglycerides. Longer reaction times did not remedy this situation, and it was decided that there was a certain probability that these species would exist regardless of extended reaction time.

Deodorization of the final oils was necessary to remove residual benzene and off-flavors. During deodorization, some material was collected in the dry ice/acetone trap of each batch. TLC analysis showed that the trap contents were composed of short-chain triglycerides (SSS, SSL or SLS), FAME, and mono- and di-glycerides. Grassy off-flavors not unlike those generated during conventional steam deodorization of vegetable oils were detected by sniffing.

Gas chromatography analyses conducted on the finished oils, as well as for milk fat and for high-oleate sunflower oil, are shown in Table 8. The composition of the BUTTER/SUN and the SIGMA/SUN oils are fairly similar with regard to the major even normal-chain fatty acids. As expected, the SIGMA/SUN oil has very little C14:0, since C14:0 is a trace component of the high-oleate sunflower oil. The oleic acid content of these oils showed a three-fold increase from normal milk fat. The total SCFA content of BUTTER/SUN and SIGMA/SUN oils were 10.5% and 15.2%, respectively. These values are within reported ranges for the total major SCFA content of milk fat (Jensen, 1992). Both finished oils had a bland taste not unlike high-oleate sunflower oil, and slightly like olive oil.

Cheesemaking

Preliminary attempts at cheesemaking using milk obtained from the Iowa State University herd were satisfactory for cheeses made from the natural milk emulsion, since these treatments could be made in one day. However, when whole milk was separated following pasteurization to give skim milk for the production of recombined cheeses, the separation process took one hour to complete This was

Table 8. Fatty acid composition of milk fat, BUTTER/SUN oil, SIGMA/SUN oil, and TRISUN 80 high-oleate sunflower oil [a]

Fatty Acid	U.S. butter [b]	BUTTER/SUN [c]	SIGMA/SUN [c]	TRISUN 80 [d]
C4:0	3.8	1.6	1.6	
C6:0	2.1	3.0	4.5	
C8:0	1.2	1.9	3.1	
C10:0	2.8	2.8	4.4	
C12:0	3.2	1.2	1.6	
C14:0	10.9	1.1	0.3	tr
C16:0	26.6	6.8	5.3	4.0
C18:0	10.8	5.6	5.9	4.0
C18:1	24.8	76.0	73.3	80.0
C18:2	2.1			10.0
C18:3	1.2			tr
C20:0				0.4
C20:1				tr
C22:0				1.0
C24:0				0.4

[a] Weight percent.

[b] Analyzed as methyl esters.

[c] Analyzed as free fatty acids.

[d] SVO Products publication.

because the separator available for use was essentially of benchtop, not pilot plant scale. The next unit operation, homogenization, took nearly two hours to complete, because of the scale of the equipment. After pasteurization, separation, and homogenization were complete, the milk had to be cooled overnight at 4°C before cheese could be made, because of time constraints. Preliminary cheeses made this way had defects such as blow holes or "yeasty" odors, indicating contamination of the milk. This is not surprising, given the processing conditions and the opportunity for contamination. It was therefore decided to use commercially available skim milk to make any cheeses that would require recombination of fat and skim milk.

The homogenization process was improved from that of Johnson (1991) because the process was continuous. This allowed for greater control of the emulsion formation. Occasionally, some portion of each batch formed a water-in-oil emulsion instead of the typical oil-in-water emulsion found in milk. When this occurred, the water-in-oil portion was rehomogenized with extra skim milk, which gave a normal emulsion.

The first set of cheeses (CONTROL1, FAT/SKIM, HOMOGCONT, BUTTERMILK, and GUM ACACIA) were made to test two major effects. First, cheese made from the natural milk emulsion that had been homogenized (HOMOGCONT) tested for the effect, if any, that the homogenization process might have on the flavor of the cheese. Earlier studies by Peters and Moore (1958a) showed that homogenization lowered fat losses in whey, reduced fat leakage from the cheese, and increased cheese yield in Swiss cheese made from milk or cream homogenized at a single-stage pressure of 170.9 kg/cm^2. These cheeses had flavor similar to those made with unhomogenized milk. When homogenization pressures were increased above 170.9 kg/cm^2, flavor, body and texture of Swiss cheese was impaired. In their review, Jana and Upadhyay (1992) summarized that cheeses made with homogenized milk may have decreased color intensity, lower pH values and higher acidity, higher proteolysis rates and accelerated ripening rates, increased moisture and fat content, and higher free fatty acid content. Despite these changes,

a homogenization step is essential in the manufacture of recombined milk cheeses (Peters, 1964; Langsrud and Reinbold, 1973a; Kebary and Morris, 1989, 1990; Metzger and Mistry, 1994).

The second effect tested in the first set of cheeses was the effect, if any, of the emulsifying agents on cheese flavor. Foda et al. (1974) reported that the addition of buttermilk solids to cheese made with recombined skim milk and milk fat did not improve final cheese flavor. Other studies have shown that the emulsification of milk fat and skim milk with gum acacia gave cheeses with superior flavor than cheeses made by simply homogenizing milk fat into skim milk (Foda et al., 1974, Johnson, 1991). Johnson (1991) also reported that the use of soy lecithin as an emulsifying agent in Swiss cheese was not as satisfactory as gum acacia.

Preliminary statistical analysis on the sensory evaluation scores for the first set of cheeses showed that there was no significant negative effect on cheese flavor from either the homogenization step or the use of gum acacia as an emulsifying agent. Cheeses made using buttermilk solids as an emulsifying agent also gave satisfactory sensory evaluation results, however, scores were lower than those for cheeses made using gum acacia. Therefore, it was concluded that homogenization and the use of gum acacia should have no effect on the flavor quality of the second set of cheeses, namely, BUTTER/SUN, SUN, and SIGMA/SUN.

All treatments in both sets of cheese were replicated, with the exception of BUTTER/SUN. One block of BUTTER/SUN was accidentally cooked at temperatures approaching 50°C for approximately 10-15 minutes. When sampled, this block of cheese had severe off-flavor defects, very lipolyzed flavor, very poor texture and body, and no eye development. These defects are similar to some described by Langsrud and Reinbold (1973), and most likely arose from several sources. Propionic acid content was close to zero, and sensory evaluation panelists reliably commented that this cheese was completely unacceptable. Although chemical, physical, and sensory data were collected for this block of cheese, the data were later omitted from statistical analyses. Therefore, values reported for the

BUTTER/SUN treatment represent repeated measures on one block of cheese. Because cheeses from both experimental sets were replicated except for the BUTTER/SUN treatment, it was decided that all chemical, physical, and sensory results would be evaluated statistically as one experiment.

Free Fatty Acid Analysis

Major Even Normal Chain Free Fatty Acids

The free fatty acid sample preparation method of de Jong and Badings (1990) was used in combination with the GC analysis method of Ceccon (1990). The methods were fast, simple, and convenient. They reliably measured free fatty acids from C2:0 - C18:1 without the need to use different analytical or preparative methods for different chain length classes. Recoveries were acceptable for all fatty acid chain lengths for each phase of sample preparation and analysis.

Acetic acid levels in this study ranged from 0.60 to 1.78 mg/g cheese, and propionic acid levels ranged from 1.15 to 2.75 mg/g cheese, as shown in Table 9. These values are within the range reported by previous investigators (Biede and Hammond, 1979; Hollywood and Doelle, 1984; Vangtal and Hammond, 1986). Acetic and propionic acids are normal metabolic products of the cultures used in Swiss cheese production, with a ratio of 1:2 acetic to propionic acid being normal (Hammond, 1994). The levels reported here indicate that normal fermentation had occurred in the cheeses. Table 9 shows that the BUTTERMILK treatment had significantly higher levels of acetic acid than most other treatments. This could be due to increased levels of lactose in the recombined fat and skim milk, as supplied by the added buttermilk solids. Values for acetic acid reported here are lower than those reported by Vangtal and Hammond (1986) for commercial Swiss cheeses. Samples with the highest propionic acid levels also had the greatest number of

Table 9. Mean free acetic and propionic acid concentrations in whole cheese [a,b]

Sample	Acetic acid (mg/g cheese)	Propionic acid (mg/g cheese)
CONTROL1	1.00 ± 0.40 [B,C]	2.75 ± 0.60 [A]
CONTROL2	1.55 ± 0.48 [A,B]	1.21 ± 0.59 [D]
HOMOGCONT	1.14 ± 0.26 [B,C]	1.15 ± 0.23 [D]
BUTTERMILK	1.78 ± 0.28 [A]	2.76 ± 0.45 [A]
GUM ACACIA	1.35 ± 0.26 [A,B]	2.20 ± 1.11 [A,B,C]
FAT/SKIM	1.01 ± 0.69 [B,C]	1.61 ± 0.39 [C,D]
SUN	1.00 ± 0.27 [B,C]	1.77 ± 0.30 [C]
SIGMA/SUN	0.70 ± 0.39 [C]	1.65 ± 0.32 [C,D]
BUTTER/SUN [c]	0.60 ± 0.13 [C]	2.57 ± 0.10 [A,B]

[a] Values with different superscripts within the same column are significantly different at $p<0.05$.

[b] Values represent averages of repeated measures from two replicate blocks of cheese per treatment, except for BUTTER/SUN, which was not replicated.

[c] *Standard deviation represents deviation of* repeated measures.

eyes.

The BUTTERMILK treatment had propionic acid levels significantly higher than those of most other treatments. Propionic acid levels were within the range reported by Vangtal and Hammond (1986) for CONTROL1, BUTTERMILK, GUM ACACIA, and BUTTER/SUN treatments. All other treatments had lower levels of propionic acid.

The free fatty acids that are liberated from milk fat in cheese through the action of enzymes make a large contribution to Swiss cheese flavor (Paulsen et al., 1980). In this study, several fat types were present in the cheeses, which can account for the range of differences in free fatty acid levels observed across the treatments. For example, cheeses made with unmodified high-oleate sunflower oil should not contain C4:0 - C12:0 free fatty acids in any appreciable level, due to the absence of these fatty acids in the triglycerides. Because all cheese treatments were included in each statistical analysis, the means groupings for free fatty analysis can be expected to be more diverse than if only natural milk fat cheeses had been studied.

With the exception of the SUN treatment, all cheeses in this study had C4:0 - C12:0 free fatty acid levels within ranges reported previously (Biede and Hammond, 1979; Woo et al., 1984; Vangtal and Hammond, 1986) for commercial cheeses, and for most of the cheeses made from recombined fat and skim milk as reported by Johnson (1991). Cheeses made from milk fat had similar C14:0 levels as reported in previous studies, yet C16:0 levels were lower than previously reported. In this study, C18:0 and C18:1 were quantified separately, while previous investigators did not separate these two species. However, if the results for C18:0 and C18:1 from this study are combined, the summed values are slightly lower than those reported by Vangtal and Hammond (1985) and Johnson (1991), but within the ranges reported by Biede and Hammond (1979).

In general, the cheeses made with normal milk fat emulsion (CONTROL) or with homogenized milk fat emulsion (HOMOGCONT), had more free fatty acids than

those in which isolated milk fat or other oils had been emulsified into skim milk, either with (BUTTERMILK, GUM ACACIA, SUN, SIGMA/SUN, BUTTER/SUN) or without (FAT/SKIM) emulsifying agents. These results are shown in Table 10. Cheeses made with the natural milk emulsion tended to have the highest flavor scores and the highest levels of short-chain free fatty acids. Cheeses with low flavor scores (SUN, SIGMA/SUN) tended to have lower levels of free short-chain fatty acids. These results support conclusions by Foda et al. (1974) and Johnson (1991) that free SCFA, though present in small amounts, are vital to good flavor development in Swiss cheese.

Minor Short-chain Fatty Acids

Several problems were encountered in the analysis of the minor short-chain free fatty acids. First, the concentration step used in sample preparation was only approximate, since it was not possible to concentrate the samples to exactly 50 μL. Therefore, results are semi-quantitative. It was difficult to determine which peaks in the chromatograms definitively represented the minor short-chain compounds of interest, because of some baseline noise at high sensitivity. Unfortunately, it was not possible to make any positive peak identifications using GC-MS, because the injection port installed in the mass spectrometer would not accommodate the Nukol® column.

Despite these problems, the chromatograms from these concentrated samples revealed peaks that were not considered to be baseline noise. Based on elution patterns and retention times of these peaks in comparison to peaks which were positively identified in the samples, and in comparison to previously published reports (Ceccon, 1990; de Jong and Badings, 1990), it is possible that isobutyric, isovaleric, isocaproic, isocaprylic, and phenylacetic acids were present in cheeses made from milk fat or modified high-oleate sunflower oil. These findings would

Table 10. Mean free fatty acid concentrations in whole cheese: C4:0 to C:18:1 [a,b,c]

Sample	C4:0 (mg/g cheese)	C6:0 (mg/g cheese)	C8:0 (mg/g cheese)	C10:0 (mg/g cheese)	C12:0 (mg/g cheese)
CONTROL1	0.180 ± 0.160 ^{A}	0.105 ± 0.093 ^{A}	0.081 ± 0.065 ^{A}	0.150 ± 0.104 ^{A}	0.191 ± 0.114 ^{A}
CONTROL2	0.050 ± 0.014 ^{C,D}	0.037 ± 0.022 ^{B,C}	0.030 ± 0.014 ^{C,D}	0.065 ± 0.011 ^{B,C}	0.073 ± 0.026 ^{B,C}
HOMOGCONT	0.163 ± 0.060 ^{A,B}	0.093 ± 0.022 ^{A}	0.069 ± 0.021 ^{A,B}	0.121 ± 0.037 ^{A,B}	0.131 ± 0.040 ^{A,B}
BUTTERMILK	0.067 ± 0.004 ^{B,C,D}	0.025 ± 0.006 ^{B,C}	0.020 ± 0.010 ^{C,D}	0.028 ± 0.019 ^{C}	0.025 ± 0.010 ^{C,D}
GUM ACACIA	0.072 ± 0.003 ^{B,C,D}	0.022 ± 0.004 ^{B,C}	0.016 ± 0.005 ^{C,D}	0.027 ± 0.004 ^{C}	0.035 ± 0.007 ^{C,D}
FAT/SKIM	0.140 ± 0.073 ^{A,B,C}	0.017 ± 0.006 ^{B,C}	0.018 ± 0.004 ^{C,D}	0.027 ± 0.031 ^{C}	0.014 ± 0.009 ^{C,D}
SUN	0.009 ± 0.002 ^{D}	undetected ^{C}	undetected ^{D}	0.006 ± 0.001 ^{C}	0.003 ± 0.003 ^{D}
SIGMA/SUN	0.056 ± 0.008 ^{C,D}	0.062 ± 0.006 ^{A,B}	0.040 ± 0.009 ^{B,C}	0.057 ± 0.023 ^{C}	0.024 ± 0.014 ^{C,D}
BUTTER/SUN [d]	0.095 ± 0.001 ^{A,B,C,D}	0.065 ± 0.001 ^{A,B}	0.020 ± 0.001 ^{B,C}	0.048 ± 0.006 ^{C}	0.017 ± 0.006 ^{C,D}

Sample	C14:0 (mg/g cheese)	C16:0 (mg/g cheese)	C18:0 (mg/g cheese)	C18:1 (mg/g cheese)
CONTROL1	0.341 ± 0.254 ^{A}	1.200 ± 0.402 ^{A}	0.275 ± 0.026 ^{A}	0.782 ± 0.660 ^{A}
CONTROL2	0.231 ± 0.095 ^{A,B}	0.554 ± 0.571 ^{B,C}	0.206 ± 0.121 ^{A,B}	0.616 ± 0.228 ^{A,B,C}
HOMOGCONT	0.198 ± 0.108 ^{A,B,C}	0.711 ± 0.204 ^{B}	0.172 ± 0.051 ^{B}	0.663 ± 0.211 ^{A,B}
BUTTERMILK	0.045 ± 0.021 ^{C,D}	0.112 ± 0.049 ^{D}	0.025 ± 0.018 ^{C}	0.110 ± 0.067 ^{D}
GUM ACACIA	0.075 ± 0.022 ^{B,C,D}	0.189 ± 0.052 ^{C,D}	0.054 ± 0.016 ^{C}	0.203 ± 0.036 ^{C,D}
FAT/SKIM	0.030 ± 0.012 ^{D}	0.060 ± 0.025 ^{D}	0.018 ± 0.014 ^{C}	0.059 ± 0.057 ^{D}
SUN	0.013 ± 0.004 ^{D}	0.020 ± 0.006 ^{D}	0.008 ± 0.023 ^{C}	0.113 ± 0.055 ^{D}
SIGMA/SUN	0.011 ± 0.005 ^{D}	0.036 ± 0.022 ^{D}	0.024 ± 0.013 ^{C}	0.363 ± 0.318 ^{A,B,C,D}
BUTTER/SUN [d]	0.016 ± 0.001 ^{D}	0.031 ± 0.008 ^{D}	0.035 ± 0.028 ^{C}	0.265 ± 0.078 ^{B,C,D}

[a] Values with different superscripts within the same column are significantly different at $p<0.05$.

[b] Values represent averages of repeated measures over two replicate blocks of cheese per treatment, except for BUTTER/SUN, which was not replicated.

[c] Limit of detection = 0.1 micrograms/mL.

[d] Standard deviation represents deviation of repeated measures.

confirm the work of Vangtal (1986), Ceccon (1990), de Jong and Badings (1990), and Ha and Lindsay (1991). Cheeses made from unmodified high-oleate sunflower oil (SUN) were not analyzed for minor short-chain free fatty acids, since they would not be expected to contain any because of their inherent lack of short-chain fatty acids (SCFA). CONTROL1 and BUTTERMILK were not analyzed for minor short-chain free fatty acids, since these samples were not readily available for this analysis.

Semi-quantitative analysis of the tentatively identified peaks is shown in Table 11. Isobutyric acid was found only in CONTROL2. Isovaleric was found in all samples, and was significantly higher in GUM ACACIA cheeses. The presence of isovaleric acid in the SIGMA/SUN cheeses could conceivably be from microbial metabolism of isoleucine. Previous studies have shown that oxidative deamination of amino acids by starter microorganisms can lead to the formation of branched short-chain fatty acids. Valine has been shown to form isobutyric acid, while leucine and isoleucine can form isovaleric acid (Nakae and Elliot, 1965). Biede et al. (1979) showed that isovaleric acid can also be produced by *P. shermanii.* Valeric acid was present in all samples; but no significant difference in levels was shown. The presence of valeric acid in SIGMA/SUN cheeses could be from impurities in the synthetic SCFA starting material. Isocaproic and isocaprylic acids were found in all samples except for SIGMA/SUN. Phenylacetic acid was significantly higher in GUM ACACIA, and was not present in HOMOGCONT, BUTTER/SUN and SIGMA/SUN cheeses.

From these data, it is not possible to determine whether some of the minor branched short-chain fatty acids (MSCFA) tentatively identified in these samples are of lipid or protein origin. The evidence of isovaleric acid in the SIGMA/SUN cheeses does not necessarily clarify this uncertainty, since levels of this acid were not significantly different than levels found in most other cheeses. Commercially available synthetic SCFA like the ones used in this study are typically obtained from the processing of tropical oils, and their distillation and purification may not be

Table 11. Mean minor short-chain free fatty acid concentrations in whole cheese [a,b,c]

Sample	Isobutyric acid [d] (µg/g cheese)	Isovaleric acid [d] (µg/g cheese)	Valeric acid [d] (µg/g cheese)
CONTROL1	ND [e]	ND [e]	ND [e]
CONTROL2	0.7 ± 0.2 [A]	10.1 + 5.9 [B]	12.9 ± 6.3 [A]
HOMOGCONT [f]	undetected [B]	13.5 ± 5.7 [B]	6.8 ± 4.5 [A]
BUTTERMILK	ND	ND	ND
GUM ACACIA [f]	undetected [B]	41.2 ± 5.7 [A]	15.0 ± 4.3 [A]
FAT/SKIM	undetected [B]	17.8 ± 5.7 [B]	15.1 ± 2.1 [A]
SUN [f]	ND [e]	ND [e]	ND [e]
SIGMA/SUN	undetected [B]	25.5 ± 6.3 [A,B]	11.5 ± 5.4 [A]
BUTTER/SUN [f]	undetected [B]	22.2 ± 5.6 [A,B]	11.7 ± 4.9 [A]

Sample	Isocaproic acid [d] (µg/g cheese)	Isocaprylic acid [d] (µg/g cheese)	Phenylacetic acid [d] (µg/g cheese)
CONTROL1	ND [e]	ND [e]	ND [e]
CONTROL2	7.3 ± 8.6 [A]	8.1 ± 6.2 [A]	0.7 ± 1.0 [B]
HOMOGCONT [f]	2.7 ± 0.2 [A]	5.0 ± 0.6 [A]	undetected [B]
BUTTERMILK	ND [e]	ND [e]	ND [e]
GUM ACACIA [f]	0.6 ± 0.5 [A]	3.3 ± 0.4 [A]	4.1 ± 0.7 [A]
FAT/SKIM	2.7 ± 1.3 [A]	2.2 ± 1.1 [A]	1.3 ± 0.0 [B]
SUN [f]	ND [e]	ND [e]	ND [e]
SIGMA/SUN	undetected [A]	undetected [A]	undetected [B]
BUTTER/SUN [f]	0.7 ± 0.1 [A]	undetected [A]	undetected [B]

[a] Values with different superscripts within the same column are significantly at $p < 0.05$.

[b] Values represent averages over two replicate blocks of cheese per treatment, except for BUTTER/SUN, HOMOGCONT, and GUM ACACIA, which could not be replicated in this analysis.

[c] Limit of detection = 0.1 ug/mL.

[d] Tentative identification.

[e] ND = not determined.

[f] Standard deviation represents deviation of repeated measures.

carefully controlled. This could lead to impurities in the form of trace component odd-chain fatty acids that can be formed from the breakdown of longer-chain fatty acids in the tropical oils.

Although the results of the MSCFA analysis are incomplete, some comparisons with other studies can be made. Ha and Lindsay (1991) identified isobutyric acid in samples of Romano cheeses ranging from 0.14 - 40.00 μg/g cheese. Levels found here are within that range. Levels of isovaleric acid reported here were near the levels found in commercial Swiss cheeses (Vangtal and Hammond, 1986, and higher than the 1.0 - 4.0 μg/g cheese range reported for Romano cheeses by Ha and Lindsay. Valeric acid levels reported by Vangtal and Hammond were lower than those reported here. Ceccon (1990) and de Jong and Badings (1990) identified but did not quantify these MSCFA in various cheese samples.

Isocaproic and isocaprylic acids were tentatively identified here at higher levels than those reported by Ha and Lindsay for Romano cheeses. Phenylacetic acid in Swiss cheeses was identified by Vangtal and Hammond, but because they did not fully resolve this compound from octanoic acid using GC; comparison with quantities reported here is not possible.

Variation in MSCFA levels within treatments between many samples was fairly high. This reflects the semi-quantitative nature of this analysis. More work is needed to refine sample preparation methods and analytical quantitation methods for MSCFA analysis. Cheeses made with natural milk fat or high-oleate sunflower oil modified by the addition of milk fat SCFA (BUTTER/SUN) tended to have better flavor than cheeses made without the addition of milk fat SCFA to high-oleate sunflower oil (SUN), or the addition of synthetic SCFA to high-oleate sunflower oil (SIGMA/SUN). This not only demonstrates the importance of SCFA in cheese flavor development, but that the possible presence of MSCFA, even though they are present in trace quantities, improves the flavor of cheeses made with natural milk fat SCFA interesterified into high-oleate sunflower oil.

Fat Content of Cheeses

Fat on a dry basis ranged from 33.95 to 48.54%, with an average deviation of 2.45%. CONTROL2 had the lowest fat content, which was well below the legal limit for fat in Swiss cheese, and significantly lower than CONTROL1. This may be because a new cream separator was used in the milk processing for CONTROL2 cheeses, and it may not have been properly set. Despite this, CONTROL2 cheeses were judged to be acceptable by sensory evaluation panelists. Other cheeses made with the natural milk emulsion (CONTROL1, HOMOGCONT) had fat contents within the 47 - 49% range reported for Iowa-style Swiss cheese (Reinbold, 1972). Cheeses made with recombined fat and skim milk, with or without emulsifying agents, all had fat contents lower than cheeses made with the natural milk fat emulsion, except for CONTROL2. This could be due to incomplete homogenization, or some emulsion breakdown during processing. Results are shown in Table 12.

Moisture Content of Cheeses

Moisture content of the cheeses ranged from 40.75 to 45.30%, which is close to, but slightly above, the 39 - 41% range for Iowa-style Swiss cheese reported by Reinbold (1972). There was no significant difference in moisture content between cheeses, as shown in Table 12.

pH of Cheeses

The pH of the cheeses ranged from 4.92 to 5.38. These values are all below the desired pH of 5.60 for Iowa-style Swiss cheese (Reinbold, 1972; Langsrud and

Table 12. % fat, % moisture, pH, and proteolysis in whole cheese [a,b]

Sample	% Fat (dry basis)	% Moisture	pH	Proteolysis (umoles glycine/ g cheese)
CONTROL1	44.15 ± 4.25 ^{A,B}	45.30 ± 2.65 ^{A}	4.94 ± 0.16 ^{E,F}	334.0 ± 31.0 ^{A}
CONTROL2	33.95 ± 0.47 ^{D}	42.74 ± 1.39 ^{A,B}	5.25 ± 0.09 ^{C,D}	152.0 ± 57.0 ^{C,D}
HOMOGCONT	48.54 ± 3.85 ^{A}	40.75 ± 2.54 ^{B}	5.07 ± 0.10 ^{D,E}	289.0 ± 15.0 ^{B}
BUTTERMILK	42.34 ± 0.67 ^{B,C}	44.38 ± 0.19 ^{A}	5.11 ± 0.06 ^{C,D}	266.0 ± 113.0 ^{A,B}
GUM ACACIA	42.67 ± 3.00 ^{B,C}	44.40 ± 3.35 ^{A}	4.92 ± 0.10 ^{F}	111.0 ± 4.0 ^{D,E}
FAT/SKIM	40.75 ± 3.97 ^{B,C}	44.88 ± 0.86 ^{A}	5.05 ± 0.01 ^{D,E,F}	41.0 ± 39.0 ^{E}
SUN	39.44 ± 2.76 ^{C}	44.96 ± 0.57 ^{A}	5.38 ± 0.03 ^{A}	167.0 ± 15.0 ^{C,D}
SIGMA/SUN	38.36 ± 2.37 ^{C,D}	43.09 ± 2.23 ^{A,B}	5.36 ± 0.04 ^{A,B}	132.0 ± 20.0 ^{D}
BUTTER/SUN [c]	41.51 ± 0.74 ^{B,C}	43.09 ± 1.32 ^{A}	5.15 ± 0.09 ^{C,D}	216.0 ± 87.0 ^{B,C}

[a] Values with different superscripts within the same column are significantly different at $p<0.05$.

[b] Values represent averages over two replicate blocks of cheese per treatment, except for BUTTER/SUN, which was not replicated.

[c] Standard deviation represents deviation of repeated measures.

Reinbold, 1973). These lower pH values could be due to inconsistent monitoring of acid development during the initial ripening period. The pH of cheeses made with high-oleate sunflower oil or modified high-oleate sunflower oil tended to be higher than cheeses made with milk fat. There were strong negative correlations between pH and sensory evaluation "acid" scores. This can be expected, since a low pH value should correspond to a high "acid" score. Results are shown in Table 12.

Proteolysis in Cheese

Protein breakdown during cheese ripening is an important factor in cheese flavor and quality (Adda, 1982; Hammond, 1994). Proteolysis produces compounds such as free amino acids and small peptides which can produce distinct Swiss cheese flavors either alone, as flavor precursors, or by interaction with other components in the cheese matrix such as α-dicarbonyls (Griffith and Hammond, 1989), and calcium or magnesium (Biede, 1977). Breakdown of amino acids can lead to the formation of certain compounds; the breakdown of valine forms isobutyrate, the breakdown of isoleucine forms isovalerate (Adda, 1982), and the degradation of phenylalanine produces phenylacetic acid (Vangtal and Hammond, 1986). Increased free amino acid content in Cheddar cheese is highly correlated with increased flavor intensity (Fox, 1989). Accelerated ripening studies with Cheddar cheese (Kristoffersen et al., 1967; Green and Manning, 1982) have also supported the importance of free amino acids and peptides in cheese flavor development.

Proteolysis in cheeses varied over a wide range from 41.0 to 334.0 μmoles glycine/g cheese. Proteolysis was lowest in cheeses made without an added emulsifier (FAT/SKIM); these levels were significantly lower than those of most cheeses made with an emulsifying agent (Table 12). Values for proteolysis in these cheeses agree with values reported by Vangtal and Hammond (1986) for 15

commercial Swiss cheeses, and by Biede and Hammond (1979a) for seven commercial Swiss cheeses.

Sensory Evaluation of Taste and Flavor

The sensory evaluation techniques used were similar to those of Biede and Hammond (1979b), except that a continuous 15 cm line scale was used to record panelists' responses. Eight flavor notes were distinguished by the panelists for the first set of cheeses, and nine for the second set of cheeses. The ninth flavor note, "lipolyzed," was included because one block of BUTTER/SUN cheeses was very lipolyzed, according to preliminary tasting.

Statistical analysis of panelist versus sample interactions showed a significant interaction for "burned" and "typical" flavor notes. An interaction would indicate that the panelists were not scoring the cheeses in the same order as other panelists for any given flavor note. When the results of one panelist were excluded from the data sets, no significant interaction was found. "Burned" and "typical" were considered by the panelists to be the most challenging flavor notes to rate. With the exception of the one panelist, the lack of a significant panelist/sample interaction indicated that the training was sufficient. Results for taste and flavor scores are shown in Table 13.

Since panelists scored the taste and flavor of the cheese in the order of perception, "sweet" was the first note evaluated. Sweetness in Swiss cheese has been attributed to proline (Hintz et al., 1956). HOMOGCONT was significantly sweeter than all other treatments, while cheeses made with high-oleate sunflower oil or modified high-oleate sunflower oil had lower scores. There was no significant difference between treatments for "salty." "Acid" scores gave a variety of means groupings, which closely followed the results for pH measurements. CONTROL1 was significantly more "acid" than SUN, SIGMA/SUN, BUTTER/SUN, FAT/SKIM,

Table 13. Mean sensory evaluation scores for taste and flavor attributes of whole cheese [a,b,c]

Sample	Sweet	Salty	Acid	Bitter
CONTROL1	5.55 ± 0.88 [B]	5.46 ± 0.59 [A]	7.58 ± 1.56 [A]	4.79 ± 0.67 [A,B]
CONTROL2	5.25 ± 0.83 [B,C]	4.47 ± 0.18 [A]	4.86 ± 0.18 [B,C,D]	3.59 ± 0.41 [A,B]
HOMOGCONT	7.05 ± 0.47 [A]	5.55 ± 0.82 [A]	6.04 ± 1.35 [A,B,C]	4.07 ± 0.26 [AB]
BUTTERMILK	5.72 ± 0.09 [B]	4.92 ± 0.10 [A]	6.60 ± 0.35 [A,B]	5.15 ± 0.67 [A]
GUM ACACIA	5.71 ± 0.36 [B]	5.48 ± 0.35 [A]	5.82 ± 0.35 [A,B,C,D]	4.97 ± 1.60 [A,B]
FAT/SKIM	4.87 ± 0.14 [B,C,D]	4.94 ± 0.63 [A]	5.20 ± 0.71 [B,C,D]	4.77 ± 0.39 [A,B]
SUN	4.01 ± 0.16 [C,D]	5.06 ± 0.77 [A]	3.95 ± 0.15 [D]	3.78 ± 0.39 [A,B]
SIGMA/SUN	3.85 ± 0.59 [D]	4.46 ± 0.30 [A]	4.27 ± 0.15 [C,D]	3.35 ± 0.35 [B]
BUTTER/SUN [d]	3.55 ± 0.67 [D]	5.61 ± 0.48 [A]	5.56 ± 0.59 [B,C,D]	4.39 ± 0.59 [A,B]

Sample	Buttery	Volatile	Typical	Burned	Lipolyzed
CONTROL1	4.28 ± 0.72 [A,B]	7.70 ± 0.59 [A]	7.49 ± 0.36 [A]	5.32 ± 0.03 [A,B]	not evaluated
CONTROL2	4.31 ± 0.67 [A,B]	5.89 ± 0.29 [A,B]	7.10 ± 0.11 [A]	3.45 ± 0.27 [D]	2.12 ± 0.07 [B]
HOMOGCONT	5.88 ± 1.27 [A]	6.67 ± 0.50 [A,B]	6.32 ± 0.78 [A,B]	4.91 ± 0.55 [A,B,C]	not evaluated
BUTTERMILK	3.89 ± 0.26 [B]	5.64 ± 0.72 [B,C]	5.49 ± 0.58 [B,C]	4.58 ± 0.31 [B,C]	not evaluated
GUM ACACIA	4.88 ± 0.24 [A,B]	6.72 ± 1.69 [A,B]	6.41 ± 0.37 [A,B]	5.65 ± 0.71 [A]	not evaluated
FAT/SKIM	4.96 ± 1.20 [A,B]	5.00 ± 0.21 [B,C]	6.10 ± 1.01 [A,B]	4.73 ± 0.02 [A,B,C]	not evaluated
SUN	3.65 ± 0.39 [B]	3.84 ± 0.40 [C]	3.36 ± 0.12 [D]	3.47 ± 0.11 [D]	2.57 ± 0.42 [A,B]
SIGMA/SUN	3.55 ± 0.57 [B]	5.37 ± 1.06 [B,C]	4.37 ± 0.99 [C,D]	3.50 ± 0.61 [D]	2.94 ± 0.33 [A,B]
BUTTER/SUN [d]	3.55 ± 0.67 [B]	6.78 ± 0.68 [A,B]	5.25 ± 0.54 [B,C]	3.97 ± 0.33 [C,D]	3.56 ± 0.27 [A]

[a] Values with different superscripts within the same column are significantly different at $p<0.05$.

[b] Values represent averages over two replicate blocks of cheese per treatment, except for BUTTER/SUN, which was not replicated.

[c] Values represent measurements, in centimeters, from sensory evaluation linescales.

[d] Standard deviation represents deviation of repeated measures.

and CONTROL2.

BUTTERMILK was significantly more "bitter" than SIGMA/SUN; this could be due to a possible increase of bitter peptides arising from proteolysis of the additional buttermilk solids present in this treatment. "Buttery" flavor, which is largely attributed to diacetyl, is an important component in Swiss cheese flavor (Langler et al, 1967; Biede and Hammond, 1979a). High levels of diacetyl in Swiss cheese, however, are associated with an immature cheese (Vangtal and Hammond, 1986), as diacetyl levels decrease over the ripening period. Panelists found that HOMOGCONT was significantly more "buttery" than cheeses made with high-oleate sunflower oil or modified high-oleate sunflower oil (SUN, SIGMA/SUN, BUTTER/SUN).

"Volatile," the oral cavity-filling flavor note, was significantly higher in cheeses made from either the natural milk fat emulsion (CONTROL1, CONTROL2, HOMOGCONT),milk fat plus gum acacia (GUM ACACIA) or high-oleate sunflower oil modified with natural short-chain fatty acids (BUTTER/SUN), than the cheese made with unmodified high-oleate sunflower oil (SUN). The SIGMA/SUN, BUTTERMILK, and FAT/SKIM treatments, however, were not significantly more "volatile" than the SUN cheeses. This suggests that the combination of gum acacia plus the natural short-chain fraction of milk fat somehow enhances this particular flavor note in recombined cheeses.

The "burned," or caramelized flavor in Swiss cheese is thought to arise from the reaction of carbonyls with amino acids (Kowalewska et al., 1985). Cheeses made with high-oleate sunflower oil or high-oleate sunflower oil modified with synthetic short-chain fatty acids had the lowest "burned"/caramelized flavor. CONTROL2 also had low "burned" flavor that was not significantly different from cheeses made with sunflower or modified high-oleate sunflower oil; the reason for this is unknown. The highest "burned" score was for the GUM ACACIA treatment, although its score was not significantly different from FAT/SKIM, CONTROL1, or HOMOGCONT.

"Lipolyzed" flavor, which arises from excessive release of short-chain fatty acids from the triglyceride molecules, was only analyzed in the second set of cheeses. The BUTTER/SUN treatment had significantly more "lipolyzed" flavor than the CONTROL2 treatment. In general, these scores are low, indicating that cheeses were not unacceptable in terms of "lipolyzed" flavor.

"Typical" is the most important flavor note analyzed in these cheeses. "Typical" has also been called "nutty" by other investigators (Vangtal, 1986; Johnson, 1991). The chemical component or components responsible for the "typical" flavor of Swiss cheese are not entirely known. Cheeses made from unmodified high-oleate sunflower oil (SUN) had the lowest "typical" flavor, and were not significantly different from cheeses made with high-oleate sunflower oil modified with synthetic SCFAs (SIGMA/SUN). Cheeses made from the natural milk emulsion (CONTROL1, CONTROL2), or with recombined isolated milk fat with or without gum acacia (FAT/SKIM, GUM ACACIA) were significantly higher than the two previously mentioned treatments. The BUTTER/SUN and BUTTERMILK treatments fell between these two groups in "typical" scores, yet were not significantly different from recombined or homogenized milk fat cheeses. Although the cheeses made with high-oleic high-oleate sunflower oil interesterified with SCFAs had the least "typical" Swiss cheese flavor, they were still considered acceptable by the panelists.

Johnson (1991) made cheeses using corn oil interesterified with syntheticSCFAs. Although direct comparisons with cheeses made by Johnson (1991) could not be made, some trends in typical Swiss cheese flavor can be seen between the two studies. In both studies, cheeses made with the natural milk emulsion had the most "typical" or "nutty" (using Johnson's terminology) flavor, followed by cheeses made from isolated milk fat recombined with skim milk using gum acacia. In the present study, cheeses made using high-oleate sunflower oil interesterified with natural short-chain fatty acids had the next highest "typical" scores, as did Johnson's cheeses made from corn oil interesterified with synthetic short-chain fatty acids. In both studies, cheeses made with all vegetable oil had the

lowest "typical" or "nutty" scores. This suggests that cheeses made with vegetable oils interesterified with short-chain fatty acids can have acceptable Swiss cheese flavor.

Sensory Evaluation of Texture

Texture was evaluated in terms of hardness and crumbliness. A high score for hardness indicated that a cheese was more hard than soft, while a high score for crumbliness indicated that a cheese was smooth and not crumbly. Cheeses made from high-oleate sunflower oil interesterified with either natural or synthetic short-chain fatty acids (SIGMA/SUN, BUTTER/SUN) were significantly harder and more crumbly than other cheeses. The BUTTER/SUN cheeses were significantly more crumbly than all other cheeses. These results, shown in Table 14, indicate that additional work is needed to improve the texture of cheeses made with modified high-oleate sunflower oil.

Factor Analysis and Multiple Linear Regression Analyses

Because many variables were studied in the analysis of the cheeses, factor analysis was performed for ease of statistical interpretation. In particular, correlation analysis was performed to determine the relationships, if any, that existed between sensory evaluation panelist scores for taste and flavor, and for chemical and physical parameters of the cheeses. Then, a model to describe the flavor of the cheeses in this study using chemical and physical data was developed using multiple linear regression.

First, taste and flavor results (excluding "lipolyzed") were normalized and analyzed using the PROC FACTOR statement and the "Promax" rotation option.

Table 14. Mean sensory evaluation scores for texture of whole cheese [a,b,c]

Sample	Hardness	Crumbliness
CONTROL1	10.18 ± 2.58 [C]	9.91 ± 1.52 [A,B]
CONTROL2	10.73 ± 1.85 [B,C]	10.76 ± 2.14 [A]
HOMOGCONT	9.99 ± 2.06 [C]	11.47 ± 1.28 [A]
BUTTERMILK	11.03 ± 1.49 [A,B,C]	10.08 ± 1.75 [A,B]
GUM ACACIA	10.68 ± 2.38 [B,C]	8.50 ± 1.66 [B]
FAT/SKIM	10.32 ± 2.32 [C]	9.82 ± 2.34 [A,B]
SUN	11.25 ± 2.00 [A,B,C]	8.68 ± 3.00 [B]
SIGMA/SUN	12.66 ± 1.84 [A]	5.93 ± 2.38 [C]
BUTTER/SUN [d]	12.18 ± 1.96 [A,B]	2.99 ± 2.40 [D]

[a] Values with different superscripts within the same column are significantly different at $p<0.05$.

[b] Values represent averages of repeated measures from one replicate block of cheese per treatment.

[c] Values represent measurements, in centimeters, from sensory evaluation linescales.

[d] Standard deviation represents deviation of repeated measures.

Only factors with an Eigen value greater than or equal to 1.00 were considered. Results of the oblique axis rotation revealed only one factor, which included all taste and flavor variables. These variables vary together, and all of the variables contribute fairly strongly to the factor. The factor components are shown in Table 15, and this factor is designated as "cheese flavor," for ease of discussion.

Studies by Vangtal and Hammond (1986) revealed 7 factors for flavor notes. This large number of flavor factors could be because Vangtal and Hammond studied 15 commercial cheeses, which probably represent a wide variation in cultures and cheesemaking procedures. They also examined 16 different flavor notes, which is twice the number evaluated here. Variation in flavor scores in Vangtal and Hammond's work could be due to effects of cultures used and processing conditions.

Chemical and physical data were normalized and analyzed in a separate factor analysis using a similar method. This analysis yielded three factors with Eigen values greater than 1.00, and are shown in Table 16. The first factor, labeled "short-chain fatty acids" included C4:0, C6:0, C8:0, C10:0, C12:0, and C18:1. The presence of C18:1 in this factor could be due to the inclusion of high-oleate sunflower oil cheeses (SUN) in the analysis. High-oleate sunflower oil has no short-chain fatty acids, yet amounts of the C18:1 free fatty acids varied in a similar manner as short-chain free fatty acids in other cheeses. Acetic acid was also present in this factor as a weak negative component. The weakness of this component in the factor could be due to low levels of acetic acid in the SIGMA/SUN and BUTTER/SUN cheeses. The second factor, called "maturity," included long-chain fatty acids other than C18:1, fat content, and proteolysis as strong components. Acetic acid was a weak positive component of this factor. The third factor, "propionic acid," included propionic acid and moisture content as strong positive components, and pH as a strong negative component. pH normally has a negative correlation with moisture content in cheese, since increased moisture content is due to whey left in the cheese. When this happens, more lactose is

Table 15. Factor components for taste and flavor of cheese

Factor Component	"Cheese Flavor"
Burned	0.856
Volatile	0.822
Typical	0.769
Acid	0.767
Sweet	0.706
Bitter	0.659
Buttery	0.563
Salty	0.523

[a] Relative contribution to factor. Values >|0.300| are significant.

Table 16. Factor components for chemical and physical properties of cheese

Factor Component	Factor 2[a] "Short-chain"	Factor 3[a] "Maturity"	Factor 4[a] "Propionic acid"
C6:0	1.000		
C8:0	0.941		
C10:0	0.877		
C12:0	0.583		
C4:0	0.719		
C18:1	0.582		
C18:0		0.792	
Proteolysis		1.000	
C16:0		0.499	
C14:0		0.792	
% Fat		0.580	
C2:0	-0.338	0.359	
% Moisture			0.993
C3:0			1.000
pH			-0.692

[a] Relative contribution to factor. Values > |0.300| are significant.

available for conversion to various acids by starter and secondary cultures (Hammond, 1995).

In this work, an attempt was made to minimize variables to one per trial. When non-treatment variables that affect chemical and physical attributes of the cheeses are properly controlled, then variation in flavor scores between treatments could be attributed to the treatment alone. In this study, control of the fat content of the cheeses and cooking temperature during processing was not optimal, and may account for some of the variation in chemical, physical, and sensory properties of the cheese.

The separation of the SCFA and LCFA into different factors could indicate a differential mechanism of fatty acid release from triglycerides between the two chain-length groups. Vangtal and Hammond (1986) also found that SCFA and LCFA fell into separate factors. Paulsen et al. (1980) showed that *S. thermophilus*, *L. helveticus*, and *P. shermanii* produce lipases that are able to hydrolyze medium- and LCFAs from Swiss cheese triglycerides. McNeill and Connoly (1989) found differential rates of lipid hydrolysis in Cheddar cheese, based on chain length of the fatty acids. They attributed these differences to the presence of different microbial lipases in the cheese. In their review, Khalid and Marth (1990) summarized findings from various studies which showed that several *Lactobacillus* sp. were able to hydrolyze SCFA from milk, cream, cheese, and synthetic triglycerides such as tributyrin. These studies suggest that enzymes of different origin are responsible for the release of certain groups of fatty acids in dairy products. Rate of release could be due to the typical sn-position of the fatty acids on the glycerol molecule.

A simple correlation analysis among the three chemical and physical component factors and the cheese flavor factor was conducted. Table 17 shows that Factor 3 ("propionic acid") did not correlate with cheese flavor, while the other factors did. Therefore, the "propionic acid" factor was not included in further statistical analyses.

Table 17. Simple correlations between cheese flavor and chemical and physical factors

Factor 1	Factor 2 "Short-chain"	Factor 3 "Maturity"	Factor 4 "Propionic Acid"
"Cheese Flavor"	0.436	0.651	0.218
significance [a,b]	0.011	0.001	0.223

[a] Significant at $p<0.05$.

[b] Not significant at $p<0.05$.

Table 18. All-way correlations between cheese flavor and chemical/physical factors [a]

Factor 1	Factor 2 "Short-chain"	Factor 3 "Maturity"
"Cheese Flavor"	0.4360	0.6510
significance	0.0112	0.0001
Factor 2		
"Short-chain"		0.6550
significance [a]		0.0001

[a] Significant at $p<0.05$.

Table 18 shows the results from a second simple correlation analysis, which was conducted to determine the interrelationship between the "cheese flavor," "short-chain," and "maturity" factors. The "maturity" factor is strongly correlated with both the "cheese flavor" and "short-chain" factors. "Short-chain" has a weaker correlation with "cheese flavor" than does "maturity." This weaker correlation could be due to the influence of CONTROL2 cheeses, in terms of their free SCFA content and flavor scores. CONTROL2 cheeses generally had high flavor scores, yet their free SCFA contents tended to be low. The stronger correlation of "maturity" with "cheese flavor" could also be influenced by fat content of the different treatments. Cheeses with lower flavor scores (SUN, SIGMA/SUN) also tended to have low fat content, while cheeses with higher flavor scores (CONTROL1, HOMOGCONT) tended to have higher fat content. However, this correlation is confounded by the fact that CONTROL2 cheeses had low fat content but high flavor scores. Strict control of the fat content during processing of cheeses made using the treatments described here could reveal a more accurate explanation of the relationship between fat content and cheese flavor.

Examination of the ANOVA table generated by the multiple regression analysis for these data (Table 19) shows that the "short-chain" factor does not make a significant contribution to the descriptive model. In other words, most of the variance in the model is explained by the components of the "maturity" factor." When the "short-chain" factor is regressed against the "cheese flavor" factor separately from the "maturity" factor, a significant relationship is shown. However, the R^2 for this model, which shows how much variability in the model is explained the variable "short-chain," is only 0.19 (Table 20). When "short-chain" is removed from the original model, a slightly more accurate model is produced, as shown in Table 21. For this model, R^2 = 0.42, meaning that 42 % of the variation in "cheese flavor" is explained by the "maturity" factor. The descriptive model equation, therefore, becomes:

Table 19. ANOVA for model 1: "cheese flavor" = "short-chain" + "maturity

Source	Degrees of Freedom	Type III Sum of Squares	Mean Square	F Value	Prob>F
short-chain	1	0.007	0.007	0.01	0.9341
maturity	1	12.461	12.461	12.18	0.0001

[a] R^2 = 0.42; Intercept = 0.023 ± 0.176; x coefficient = 0.672 ± 0.193.

Table 20. ANOVA for model 2: "cheese flavor" = "short-chain" [a]

Source	Degrees of Freedom	Type III Sum of Squares	Mean Square	F Value	Prob>F
Model	1	10.11	10.11	7.37	0.0112
Error	31	43.15	1.39		
Total	32	53.27			

[a] R^2 = 0.19; Intercept = 0.036 ± 0.205; x-coefficient = 0.439 ± 0.163.

Table 21. ANOVA for model 3: "cheese flavor" = "maturity" [a]

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob>F
Model	1	22.57	22.57	22.79	0.0001
Error	31	30.70	0.99		
Total	32	53.27			

[a] R^2 = 0.42; Intercept = 0.023 ± 0.173; x-coefficient = 0.683 ± 0.143.

"Cheese Flavor" = (0.683) ("maturity") + 0.023

Here, "maturity" = C18:0 + proteolysis + C16:0 + C14:0 + % fat + C2:0, as shown in the factor composition given in Table 17. According to this model, measurements of proteolysis, % fat, acetic acid, and LCFAs are able to describe 42 % of the variability in these cheese. Examination of chemical and physical data reveals that cheeses with high degrees of proteolysis generally have higher fat content and flavor scores. Exceptions to this trend are the CONTROL2 cheeses, which have low fat content and degree of proteolysis, but high flavor scores. CONTROL2 cheeses also had low levels of SCFA, and this could influence the strength of the correlation of "short-chain" with "cheese flavor."

The revelation that the two chemical and physical factors, "maturity" and "short-chain," are correlated with one another is not unreasonable. Both contribute to the descriptive model, but the "short-chain" factor only contributes in a minor way. Because the two factors are correlated with one another, the idea that both fat-soluble and water-soluble components of Swiss cheese are important in flavor development is strengthened. In the "short-chain" factor, butyric acid (C4:0), and to a lesser extent caproic acid (C6:0), are water-soluble, while the other factor components are fat-soluble. In the "maturity" factor, acetic acid and products from proteolysis are water-soluble, and the LCFA are fat-soluble.

The fat-aqueous interface in cheese is thought to be important in flavor development (Foda et al., 1974). In normal milk emulsions, the milk fat globule membrane (MFGM) is the interfacial material (Jensen et al., 1991). At this interface, a variety of reactions such as lipolysis can occur (Hammond, 1994); the products of reaction are then distributed in the two phases in a controlled manner (Jensen et al., 1991). Results in this study, although not significant, suggest that the fat-aqueous interface influenced the amount of lipolysis that occurred. Cheeses made with the

natural milk fat emulsion generally showed a trend toward increased lipolysis, although this was not always significant. It is possible that the MFGM provides a better fat-aqueous interface than gum acacia, buttermilk, or no emulsifying agent, for a variety of flavor-related enzymatic reactions to occur.

The balanced component theory states that good cheese flavor requires a proper balance between a variety of compounds in the cheese (Mitchell, 1981; Adda et al., 1982). Results of this study seem to confirm this theory, since several differentially soluble components varied together, and can be used to describe the flavor of Swiss cheeses made in a variety of ways.

CONCLUSIONS

1. A reliable method for the determination of α-dicarbonyls in Lactobacilli MRS medium has been developed. This method is applicable for α-dicarbonyls in simple matrices; the extraction step may need to be modified slightly for α-dicarbonyl quantitation in more complex matrices such as cheese. Absorbance of quinoxaline derivatives is maximized at 315 nm. Interference by dihydroxyacetone is avoided using HPLC conditions outlined here.

2. Temperature control during cheese processing is critical. Viability of *Lactobacillus bulgaricus* AR2 *in vitro* is decreased if subjected to temperatures above normal processing temperatures. Production of propionic acid by *Propionibacterium shermanii* P13 was severely compromised when proper cooking temperature was exceeded; the resulting cheese had poor flavor and texture.

3. There was little evidence of reduced cell viability, and no evidence of decreased production of diacetyl, by *Lactobacillus bulgaricus* AR2 in the presence of 5.0 *mM* oleic acid. Glyoxal and methylglyoxal production was not affected by the presence of 5.0 *mM* oleic acid in the growth medium. This suggests that oleic acid in Swiss cheese, especially in cheeses made from recombined oils that are high in oleic acid, should not inhibit normal production of α-dicarbonyls, or significantly affect starter culture cell growth.

4. Isolation of the SCFAs from milk fat resulted in some mechanical losses that were difficult to avoid. More losses, in the form of mono- and diglycerides, and FAME, occurred during interesterification with high-oleate sunflower oil. The greatest losses of SCFA in the form of short-chain triglycerides occurred during

deodorization. These processes still need to be optimized to reduce such losses. Nonetheless, the resulting oils had short-chain composition not exceedingly different from natural milk fat.

5. Free fatty acids in cheese can easily be analyzed by using methods described here. Acetic and propionic acid levels were normal in all cheese types, indicating that the use of recombined oil in cheesemaking does not adversely affect acid development by starter cultures.

6. Free fatty acids released from cheese triglycerides by enzymatic means fall into two groups, as revealed by factor analysis. C4:0 - C12:0 and C18:1 free fatty acids form one group whose levels vary together, while C14:0 - C18:0 free fatty acids form a second group whose levels in cheese vary together. This suggests that more than one source of lipase acts in the cheese, as postulated earlier by Vangtal and others.

7. Minor short-chain branched and unsaturated fatty acids play a role in cheese flavor, as shown in the emulsion studies. More work is needed to effectively identify and quantify these acids in Swiss cheese.

8. The pH of these cheeses varied and was lower than optimal. Close control of moisture content during the cooking stage, and thus availability of lactose for fermentation to acetic and propionic acids, could reduce this variation.

9. Fat content of the cheeses was slightly lower than normal; yet, this did not affect acceptability of the cheeses by panelists. Interestingly, one set of control cheeses with extremely low fat content had very high sensory evaluation scores. This indicated that these cheeses could be purposefully made with reduced fat and still have acceptable flavor.

10. Proteolysis in the cheeses varied considerably. Degree of proteolysis is the major component of the model developed to describe sensory evaluation responses for cheese flavor. Other components of this model include long-chain fatty acids, fat content, and acetic acid. This model can describe 42% of the variability in Swiss cheese flavor, as determined by sensory evaluation.

11. Sensory evaluation scores for flavor varied together, as revealed by factor analysis.

12. SCFA are important in typical Swiss cheese flavor development, even though they are present in small amounts. Cheeses with the poorest flavor tended to have the lowest levels of SCFA. Perhaps starting out with greater amounts of SCFA in the synthesis of BUTTER/SUN or SIGMA/SUN oils would eliminate this problem, since losses occurred during synthesis. It is possible that SCFA release from these randomized oils did not occur as readily as from natural milk fat. Future work could employ a lipase in directed synthesis to attach SCFAs to their natural sn-1 and sn-3 positions of the glycerol molecule.

13. The natural milk fat emulsion probably plays a role in encouraging the lipolysis of cheese fat, and thus development of flavor. The fat-aqueous interface seems important in lipolysis. Gum acacia is a reasonable substitute for the milk fat globule membrane in cheeses made with recombined fat and skim milk. Homogenization does not have an effect on cheese quality.

14. It is possible to make Swiss cheese with acceptable flavor characteristics from recombined skim milk, gum acacia, and isolated milk fat or high-oleate sunflower oil modified by the inclusion of natural or syntheticSCFAs. This work suggests that cheeses made in this way can be acceptable to consumers. The texture of cheeses made with modified high-oleate sunflower oil, however, is

compromised. More work is needed to improve the texture of cheeses made from recombined fat and skim milk.

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