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The modification and use of high-oleic sunflower oil in the production of a ripened Swiss cheese-like product with good flavor quality

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ABSTRACT

Milk fat is considered hypercholesterolemic because it contains cholesterol and a large proportion of saturated fatty acids. The use of vegetable oil to substitute for milk fat in cheesemaking has been proposed to improve the nutritional value of the cheese. However, the quality of vegetable-oil cheese has not been as good as that of traditional cheese. The objectives of this study were to modify vegetable oils to resemble milk fat for use in cheese production and to produce high quality, nutritious Swiss cheese from modified vegetable oils and skim milk.

High-oleic sunflower oil (HOSO) was chemically modified by incorporating short-chain fatty acids (C4-C10) (SCFA) through interesterification of short-chain triglycerides and HOSO. A simplified gas chromatography method based on decyl esters was used to determine the fatty acid compositions of milk fat and modified vegetable oils. All modified vegetable oils had a short-chain fatty acid composition closely resembling that of milk fat. Seven types of Swiss cheese were made by recombining skim milk with various fat sources, namely, HOSO; milk fat; randomized milk fat; HOSO with commercial short-chain fatty acids interesterified at 100% and 120% of the levels in the milk fat; HOSO with SCFA from milk fat interesterified, and HOSO with free fatty acids (C4-C10) dissolved. The quality of Swiss cheese was evaluated after three months of ripening by sensory, chemical, and physical methods. All cheeses made from modified HOSO had significantly higher scores in typical Swiss flavor and volatiles than did unmodified HOSO and were not significantly different from milk fat control cheeses. Simple correlation of sensory

flavor and chemical parameters indicated that Swiss flavor was correlated positively with sweetness, volatiles, caramelized flavor, non-fat solids, and C4-C10 fatty acids and negatively with fat and salt content. A linear regression model ($R^2 = 0.91$) was established for Swiss flavor that included fat, salt, titritable acidity, and C12-16 fatty acids as variables.

Instrumental texture profile analysis indicated no differences among the treatments in texture attributes except for cohesiveness; however, sensory panelists detected differences among the treatments in hardness and springiness. The commercial feasibility of production of Swiss cheese from modified HOSO is also discussed.

CHAPTER 1. GENERAL INTRODUCTION

Introduction

Cheeses are rich in milk fat and popular among American consumers. The consumption of cheese is increasing steadily at ~ 3% annually in most countries (1). In the U.S. per capita consumption of cheese is now about 28 pounds per year (2). Although cheeses are a good source of protein and calcium, the high fat content in cheese has caused nutritional concern. Milk fat is considered hypercholesterolemic because of its high content of saturated fatty acids, especially lauric, myristic and palmitic acids (3). The consumer's demand for healthy and nutritionally balanced products has led to the development of a number of fat-free and low-fat cheese products. But, so far, it has been impossible to produce good quality cheeses without using milk fat or simulated milk fat. Vegetable oils have long been used to substitute butterfat for the manufacture of cheeses (4, 5). There are several advantages of using vegetable oils in place of butterfat in cheesemaking. The vegetable oils are cholesterol free, higher in unsaturated fatty acids, cheaper than milk fat, and some are more stable and easily stored (6, 7). However, vegetable oil cheeses were reported to have an oily off-flavor, less volatile fatty acids (8), lower sensory scores, and a soft and crumbly texture (6, 9, 10, 11).

One of the unique features of bovine milk fat is the presence of short-chain fatty acids. These short-chain fatty acids in milk fat are believed to be an important source of cheese flavor (12, 13, 14). Our hypotheses were: 1) A lack of short-chain fatty acids in vegetable oil is one of the reasons that vegetable oil cheese has inadequate flavor compared

with milk fat cheese, and 2) Incorporation of short-chain fatty acids into vegetable oils will help to improve the flavor of vegetable oil cheese. Because fatty acids with chain lengths up to C10 are digested differently than C12-C18 fatty acids and have no effect on the plasma cholesterol (15, 16), the incorporation of short-chain fatty acids (C4-C10) into long-chain vegetable oils would help to improve the flavor of vegetable oil cheese without entailing any nutritional disadvantages. Whitehouse (17) and Johnson (18) made Swiss cheese from vegetable oils that had been modified by interesterifying short-chain fatty acids (C4-C12). Their results showed great promise of making good quality Swiss cheese with modified vegetable oils. Their studies also revealed several problems that needed to be overcome before a wholly satisfactory product can be achieved. Therefore, the objectives of these studies were to modify vegetable oils to resemble milk fat for use in cheese production and to find out the optimal processing conditions for producing high quality Swiss cheese from modified vegetable oils and skim milk and to determine the sensory, chemical, and physical properties of cheeses. Seven types of Swiss cheese were made by recombining skim milk with various fat sources, namely, high-oleic sunflower oil (HOSO); milk fat; randomized milk fat; HOSO with commercial short-chain fatty acids (C4-C10) (SCFA) interesterified at two levels, 100% and 120% of the levels in the milk fat; HOSO with SCFA from milk fat interesterified, and HOSO with free fatty acids (C4-C10) dissolved. The sensory, chemical, and physical characteristics of cheese produced from modified vegetable oils were compared with those of controls made from HOSO, milk fat and randomized milk fat emulsified into the skim milk under similar

homogenization conditions. Furthermore, the possibility of making cheese from skim milk and HOSO with certain amounts of SCFA dissolved was explored.

Dissertation Organization

This dissertation contains a general introduction, including an introduction and a literature review, followed by two papers and a general conclusion. The papers are in the required format of the journals to which they will be submitted.

Literature Review

Health impact of cheese

Cheese is an excellent energy food. It is tasty, highly digestible (up to 99%), and suitable for almost all age groups. Cheese is good source of high quality protein. The protein content of cheese varieties varies between 20 and 35% (1). Like milk, the calcium and phosphorus contents of cheese are relatively high. A serving of cheese contains about 25% and up to 42% of the recommended dietary allowance of calcium (19), and cheese is an important source of vitamins and many trace elements. Many cheeses contain 20-30% fat (1), and the high fat content in cheese has caused nutritional concern. Dietary fat, especially milk fat has been linked to coronary heart disease (20, 21) because milk fat contains cholesterol and a large proportion of saturated fatty acids (3). Saturated fatty acids C12-C16 increase the concentration of atherogenic low-density lipoproteins (LDL) in blood plasma, but SCFA have no effect on LDL-cholesterol levels (15). Stearic acid does not raise cholesterol levels (22) and may decrease it (23). Unsaturated fatty acids have a

LDL-cholesterol lowering effect (16, 24, 25, 26). Polyunsaturated fatty acids (PUFA) may be more effective in lowering LDL-cholesterol than monounsaturated fatty acids (25, 26). The effects of dietary cholesterol on plasma lipoprotein are controversial. Human response to dietary cholesterol is quite variable, but dietary cholesterol makes a significant contribution to atherogenic lipoproteins in some individuals (27). Individual variation may be linked to the efficiency of cholesterol absorption from the intestine (28) and genetic traits (29, 30). The interaction between cholesterol and saturated fat intake (27) provides a further reason to limit dietary cholesterol. The current Dietary Guidelines for Americans recommend consumption of no more than 30% of total dietary energy from fat, no more than 10% from saturated fatty acids and no more than 300 mg of cholesterol per day (31). Because of the relationship between consumption of fat and cholesterol and coronary heart disease (32), the World Health Organization has recommended reduction in their consumption.

Development of nutritional cheese products

In Americans, dairy products contribute 15-20% of total fat intake, 25-33% of saturated fat intake, and about 15% of dietary cholesterol (33). In response to consumer demand, the food industry has been developing reduced and nonfat products to help meet dietary goals of reducing fat and cholesterol. Because of its high fat content, cheese is a prime target for fat reduction, and low-fat and non-fat cheeses comprised 21.2% of the total cheese sales in 1997 (2). It is easy to decrease or remove the fat from milk, but it is difficult to maintain good flavor and texture in low- or non-fat cheese (34), which often

exhibit firm, elastic or doughy textures and bland or bitter flavors (35). In Cheddar cheese, reducing the fat more than 33% gave unacceptable flavors and physical properties (34). Manipulation of cheesemaking conditions (34) and use of various additives (36, 37, 38) have been advocated for improving low-fat cheeses' sensory properties.

Fat mimetics and substitutes have been used in cheesemaking to reduce the nutritionally available fat. These include 'Olestra', a sucrose fatty acid polyester (Procter & Gamble, Cincinnati, OH) (39, 40, 41); medium-chain triglyceride (MCT) (42); 'Salatrim' (Nabisco Foods group, East Hanover, NJ), a triglyceride containing both short-chain and long-chain fatty acids (43, 44, 45); 'Dairy Lo' (Pfizer Inc., Groton, CT), 'Simplesse' (NutraSweet Co., Deerfield, IL), and 'Novagel' (FMC Corp., Philadelphia, PA) (39, 46). Some of these products have been reported to have acceptable texture, but it is unlikely that they will yield cheeses with acceptable ripened-cheese flavors.

Imitation cheese formulated from vegetable oil, protein, and water also are marketed. The proteins in the imitation cheeses are typically a casein/caseinate combination, but isolated soy protein is also being used in some formulations (47). In the U.S., the sales of imitation cheese represents only approximately 2% of the total cheese sale in 1997, but the sales increased 14.5% compared to a year ago (2). Process cheeses simulating Mozzarella, Cheddar, and Gouda account for most of imitation cheeses. Imitation process cheeses often give a pleasant acid taste but generally are without typical cheese flavors (48).

Whitehouse (17) and Johnson (18) made Swiss cheeses by using modified corn oil and high-oleic sunflower oil, respectively. The vegetable oils were modified by

interesterifing short-chain fatty acids (C4-C12) into them in order to improve the flavor of vegetable oil cheeses. In both studies, cheese made with the natural milk fat emulsion had more "typical" or "nutty" flavors than other cheeses, and cheeses made with unmodified vegetable oils had the lowest "typical" or "nutty" scores. Cheeses made with gum acacia and modified vegetable oils had a nutty flavor intensity slightly lower than that of cheese made from butter oil and gum acacia. Both studies suggested that cheese flavor was improved by using vegetable oils interesterified with short-chain fatty acids. Whitehouse (17) noticed that the texture of modified vegetable oil cheeses was significantly harder and more crumbly than other cheeses. Cheese texture was not evaluated in Johnson's study (18). The poor texture of Whitehouse's cheese might be attributed to insufficient homogenization of skim milk with fats and the large fat loss during the manufacture of the cheeses (17).

Free fatty acids and cheese flavor

One of the unique features of bovine milk fat is the presence of short-chain saturated fatty acids. The normal, saturated free fatty acids with four- to ten-carbon chain lengths seem to have the most flavor potential. Fatty acids longer than lauric have little taste or aroma, but they may give soapy mouth sensations if the concentration is high enough (49). Fatty acids in cheese mostly originate from the lipolysis of the milk fat and contribute to cheese flavors, especially in hard Italian cheeses, such as Romano, Parmesan, and Provolone, in mold-ripened varieties (12), and in Cheddar cheese (13). Woo et al. (14) quantified major free fatty acids in several cheese varieties and confirmed that C4 and C6

free fatty acids are important to flavors of aged Gruyere as well as Swiss cheese. They also emphasized the importance of branched short-chain fatty acids from amino acid degradation and phenolic fatty acids from feed in cheese flavors. Vangtal and Hammond found that the free fatty acids shorter than C10 and longer than C10 varied as two independent groups in Swiss cheeses (50). Bills and Day (51) found only small differences between the concentration of individual free fatty acids in Cheddar cheeses of different flavor, and the proportions of fatty acids C6:0 to C18:3 were similar to those in milk fat, suggesting that these fatty acids were released non-specifically. However, free C4:0 fatty acid was found at higher concentration than could be explained by its proportion in milk fat.

Low-molecular weight fatty acids (C2-C10) in cheese and cheese curd slurries are not necessarily formed by the lipolysis of milk fat but also formed during the ripening of cheese containing vegetable oil as a replacement for milk fat (52). Cheddar and Romano slurries with a vegetable oil-skim milk system produced little flavor. Barlow et al. (53) found the concentration of butyric and hexanoic acids correlated with Cheddar flavor, and the best Cheddar flavor was associated with 45-50 mg/kg butyric acid and 20-25 mg/kg hexanoic acid (53). Perret (54) observed that the addition of low-molecular weight fatty acids to commercial Cheddar curd, followed by a period of maturation, had a much greater effect on flavor than similar additions to previously matured cheese. It is thus possible that metabolites of fatty acids rather than the fatty acids themselves are important for Cheddar flavor. Kristoffersen et al. (55) reported that partially hydrogenated soybean oil and 85% soybean oil-15% cottonseed oil blends served as a suitable replacement of milk fat for

Cheddar-type cheese, but attempts to make Romano-type cheeses using the same vegetable oils resulted in poor, unacceptable flavor since short-chain fatty acids are more essential to the flavor of Romano than of Cheddar cheese. Foda et al. (56) made Cheddar cheese from various fats homogenized into skim milk. Milk fat homogenized into skim milk gave better flavor than other fats but was inferior to the natural milk emulsion.

Modification of vegetable oils to resemble milk fat

Most vegetable oils contain no short-chain fatty acids. Since short-chain fatty acids are important in cheese flavor, modification of vegetable oils with SCFA should improve the flavor of vegetable-oil cheese. There are many ways to incorporate SCFA into vegetable oils. Short-chain triglyceride (SCTG) can be synthesized from free fatty acids and glycerol using acids as catalysts. Benzene azeotrope distillation has been a traditional method used to remove water from such esterification systems (57). This process is not suitable for preparing edible material because benzene is considered a carcinogen (58). Toluene has chemical and physical properties similar to benzene, but toluene is less toxic (59). Toluene appears not to be carcinogenic or mutagenic, and no substantiated evidence has been found of adverse effects on reproduction or teratogenicity (60). Using toluene instead benzene to remove water in the synthesis of SCTG seems acceptable.

Both chemical and enzymatic methods have been used to conduct the interesterification of SCTG with long-chain vegetable oil. During chemical-catalyzed interesterification, all fatty acids of the parents oils are randomly rearranged until a wholly random distribution is finally achieved (61, 62, 63). The most widely used catalyst is

sodium methoxide. Sodium methoxide solution in methanol or a powdered sodium methoxide are commercially available. The various forms of sodium methoxide are easy to handle, inexpensive, and the reaction is normally conducted at 50-70°C. Sodium methoxide is used at a range of 0.2 - 2% of the oil weight. After reaction, the catalyst can be easily removed by a water wash. But the quality of sodium methoxide varies widely with exposure to air and water vapor. After the addition of the catalyst, there is usually an induction period before the reaction sets in, and there is a loss of oil due to the formation of soap and methyl esters (63).

The synthesis of SCTG also can be achieved by using enzyme-catalyzed esterification. Kosugi and Azuma (64) synthesized triglyceride from polyunsaturated fatty acids (PUFA) by immobilized lipase from *Candida antarctica* or *Rhizomucor miehei*. Polyunsaturated fatty acid ethyl esters were also used as the substrates. Pure triglycerides were isolated from polar fatty acids, monoglycerides, and diglycerides by passing the product through an aluminum oxide column. With ethyl esters as the substrate, the residual ethyl ester was difficult to remove by aluminum oxide or silica gel. Glycerol could be in 10% excess over the stoichiometric amount to eliminate the residual ethyl esters, but this would generate mono- and di-glycerides rather than triglycerides. Thus, the enzymatic method involves a time-consuming column purification step and was more costly than the chemical method.

Short-chain fatty acids could be incorporated directly into long-chain vegetable oil by lipase-catalyzed acidolysis (65). Since a relatively high percentage of free fatty acids must be incorporated, a great amount of free fatty acids would be generated, and these

acids would have to be removed by high temperature deodorization or some other technique. Fatty acid ethyl esters (65) or fatty acid methyl esters (66) could also be used as acyl donors in the lipase-catalyzed transesterification.

Enzymatic interesterification is a recent development of considerable promise. The advantage of enzymatic interesterification over the chemical procedures lies in the additional control of product composition. The lipase, coated onto a support material (kieselguhr, hydroxyapatite, alumina) in the presence of a little water, supports interesterification at about 40°C and usually require 16-70 h for the complete reaction. The process may be operated in a batch or continuous manner (67). Enzymes of various specificities allow some control of the reaction (68).

Randomization of butter fat

More than 400 different fatty acids in bovine milk fat have been identified (33). In natural milk fat, fatty acids are not distributed randomly on the glycerol (Table 1) (33). Butyric and other short-chain fatty acids are mainly distributed on the *sn*-3 position. Randomization of butterfat has been carried out by using sodium methoxide as a catalyst (69, 70, 71). Through randomization, short-chain fatty acids were distributed more randomly on the glycerol. Kuksis et al. (70) reported that randomized butter fat had a setting point 5.5°C higher than did normal butter fat. Weihe (71) found that a randomized, steam-deodorized butterfat had an acceptable flavor.

Table 1. Positional distribution of fatty acids in milk fat (mol %) (33)

Fatty acid	*sn*-1	*sn*-2	*sn*-3
4:0	-	-	35.4
6:0	-	0.9	12.9
8:0	1.4	0.7	3.6
10:0	1.9	3.0	6.2
12:0	4.9	6.2	0.6
14:0	9.7	17.5	6.4
16:0	34.0	32.3	5.4
18:0	10.3	9.5	1.2
18:1	30.0	18.9	23.1
18:3	1.7	3.5	2.3

Effect of homogenization and emulsifying agents on cheese quality

Usually, the purpose of homogenization in the dairy industry is to reduce the size of the fat globules and change the milk fat globule membrane (72). Homogenization makes it possible to make cheese from recombined milk. Homogenization of milk or cream has been reported to improve the overall sensory quality of certain types of cheese and to increase cheese yield (73), but high homogenization pressures impair the body and texture of Swiss and Mozzarella cheeses (73). High pressures give increased smoothness in white cheese and increased moisture retention in high moisture verities such as Feta (74). Czulak et al. (75) recommended using pressures not exceeding 70.4 kg/cm^2 (single-stage) and 140.8 + 35.2 kg/cm^2 (two-stage) respectively in the production of Cheddar cheese from recombined milk. Abd El-Salam and El-Shibiny (76) found that varying the homogenization pressure from 100 to 250 kg/cm^2 in the manufacture of recombined pickled soft cheese had little effect on composition and cheese quality, but affected yield. Law et al. reported that cheese made from skim milk and milk fat was indistinguishable from whole milk Cheddar cheese if the milk fat was homogenized into the skim milk at low pressures so that lipolysis did not occur (77). Flavor development has been reported to be slower in recombined Gouda (78), Ras (79), and Domiati (80) cheeses than in those made from fresh milk. The concentration of free fatty acids, soluble nitrogenous substances and free amino acids were lower in recombined Domiati and Gouda cheeses. Whitehouse (17) and Johnson (18) both found Swiss cheese made from natural milk emulsion had more intense "typical" or "nutty" flavor than cheese made from recombined

milk fat and skim milk, but the differences were not significant when gum acacia was used as an emulsifier. So, they concluded that homogenization had no effect on cheese quality.

Several emulsifiers have been used in the production of recombined milk cheese. Incorporation of lecithin in the recombined milk prior to homogenization was beneficial in the manufacture of Feta, Halloumi, and Mozzarella cheeses (81, 82). Use of buttermilk solids in the manufacture of recombined pickled soft cheese improved its yield, moisture, and flavor intensity (83). Foda et al. (56) reported that the addition of buttermilk solids to cheese made with recombined skim milk and milk fat did not improve final cheese flavor. Both Johnson (18) and Foda et al. (56) found that the homogenization of milk fat and skim milk with gum acacia as an emulsifier gave better cheese flavor compared with simply homogenizing milk fat into skim milk. Johnson (18) also reported that the use of soy lecithin as an emulsifier in Swiss cheese was not as satisfactory as gum acacia. Whitehouse (17) used both gum acacia and buttermilk solids as an emulsifier in the production of recombined Swiss cheese and found both emulsifiers gave satisfactory sensory results, but using gum acacia as an emulsifier resulted in better flavored cheese than buttermilk solids.

Milk fat fatty acid analysis by gas chromatograph

The fatty acids in milk fat represent a wide range of carbon chain lengths, and this makes the accurate analysis of the fatty acids in milk fat difficult. Traditionally, triglycerides are converted to methyl or butyl esters, followed by gas chromatography (GC) (84). The short-chain fatty acids, especially butyric acid, determined by these

methods might be underestimated since short-chain fatty acids are subject to loss during the analysis because of their volatility. Butyl esters usually provide a more reliable means of analysis than methyl esters (85). A direct capillary GC method was reported by de Jong and Badings (86) and Whitehouse (17) for fatty acid analysis in milk and cheese, making the tedious procedure for converting fatty acids to their derivatives unnecessary. However, the long-chain fatty acids (C16-C18) gave a lower response than C12 in their analysis. Due to the temperature limitation of the column used, the direct GC method was not good for analysis of fats that contain fatty acids with chain length longer than C18. Vangtal and Hammond (50) converted fatty acids to their decyl ester derivatives for GC analysis. This method gave good recovery for both short chain and long chain fatty acids, but involved a time-consuming thin-layer chromatography (TLC) step to separate the excess decanol from the decyl esters before the GC analysis.

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CHAPTER 2. MODIFICATION OF VEGETABLE OIL FOR USE IN CHEESE PRODUCTION

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Abstract

The objectives of this study were to modify vegetable oils to resemble milk fat for use in experimental cheese production and to develop a satisfactory method for fatty acid analysis of milk fat, vegetable oils modified with short-chain fatty acids, and cheese fat. Short-chain triglyceride (SCTG) was synthesized by esterifying short-chain fatty acids (SCFA) with glycerol, and using a toluene azeotrope to remove the water of esterification. SCFA from two sources were used: 1) commercial acids and 2) acids isolated by double distillation of milk fat methyl esters. SCTG was incorporated into high-oleic sunflower oil (HOSO) at 100% and 120% the levels in the milk fat, by sodium methoxide-catalyzed interesterification. For fatty acid analysis, milk fat or other fats that resemble milk fat were converted to their decyl ester derivatives and the transesterification mixtures were injected into a gas chromatograph directly without further purification. The method was accurate and fast for fatty acid analysis of fats that contain a wide range of fatty acid chain lengths. The SCTG synthesized from milk fat methyl esters had a better color than those made from commercial acids and required no bleaching. The SCTG had a bitter, unacceptable flavor, but after interesterification into HOSO and deodorization, the flavor was quite acceptable.

All modified HOSOs gave bland and acceptable flavors and had a short-chain fatty acid composition close to that of milk fat.

Key words: High-oleic sunflower oil, vegetable oil, oil modification, interesterification, short-chain triglyceride, milk fat, cheeses.

Introduction

Milk fat is a major component in many cheese varieties, and with the increased nutritional concern over the saturated fatty acid and cholesterol contents of fats, many people limit their consumption of cheese. Milk fat is considered hypercholesterolemic because of its high content of saturated fatty acids (70%), especially lauric, myristic and palmitic acids (1). Physical, chemical, and biological processes have been used to modify the composition of milk fat. From a nutritional standpoint, a suggested ideal milk fat would contain 10% polyunsaturated fatty acids, 8% saturated fatty acids, and 82% monounsaturated fatty acids (2). Feeding cows with a source of long-chain or highly unsaturated fatty acids can be used to make milk fat nutritionally more desirable (3, 4, 5), and the maximal extent of such fatty acid alteration by diet manipulation has not been determined. But the impact of this research will be limited until there are incentives for producers to feed special diets (4).

Vegetable oils with fatty acid compositions resembling that proposed as ideal milk fat are commercially available. High-oleic sunflower oil (HOSO), for example, typically contains 9% polyunsaturated fatty acids, 10% saturated fatty acids, and 81%

monounsaturated fatty acid. But HOSO has no short-chain fatty acids, and the short-chain fatty acids in milk fat are believed to be an important source of cheese flavor (6, 7). Studies also indicated that fatty acids with chain lengths up to C10 are digested differently than C12-C18 fatty acids, and have no effect on the plasma cholesterol (8, 9). Therefore, incorporation of short-chain fatty acids (C4-C10) into long-chain vegetable oil could improve the flavor without entailing any nutritional disadvantages.

Whitehouse (10) and Johnson (11) used a benzene azeotrope distillation step to synthesize SCTG from free fatty acids. This process is not feasible as a commercial process because benzene is considered a carcinogen (12). In this study, the possibility of using toluene instead of benzene to remove water from the reaction system was explored since toluene is considered less toxic (13, 14, 15, 16). SCTG can be incorporated into long-chain vegetable oils by sodium methoxide-catalyzed interesterification of short- and long-chain fatty acid triglycerides (10, 11).

Milk fat is distinctive for its wide range of chain lengths. The analysis of milk fatty acids by gas chromatography (GC) is difficult because of the potential loss of short-chain fatty acids during analysis. The problem was overcome by Vangtal (7) by converting the fatty acids to their decyl esters derivatives. But this method involved a time-consuming thin-layer purification step. Therefore, the objectives of this study were 1) to modify vegetable oil to resemble milk fat so the modified oil could be used in high quality cheese production and 2) to develop an improved method for fatty acid analysis of milk fat or simulated milk fats.

Experimental Procedures

Milk fat preparation. USDA Grade AA sweet cream butter (Prize of Iowa, Mid-America Farms, Springfield, MO) purchased from a local grocery store was melted in a 60°C-oven, and centrifuged in 250-ml centrifuge bottle for 20 min at 1900x g. After centrifugation, the bottle was again placed in a 60°C-oven to liquefy all the fat, and the clear oil phase was recovered. The fatty acid composition of pooled butterfat was determined by GC.

Preparation of milk fat fatty acid methyl esters. Milk fat was converted to methyl esters by reacting milk fat with 130% of the theoretical amount of methanol and sodium methoxide (5.4 M sodium methoxide in methanol, Fluka Chemical Co., Ronkonkoma, NY) at 1% weight of fat in a sealed container at 40°C under vigorous stirring overnight. The reaction mixture was washed with warm water until the washings were neutral and clear. The methyl esters were dried over anhydrous sodium sulfate (Fisher Scientific, Fair Lawn, NJ).

Isolation of short-chain fatty acids from milk fat methyl esters. The SCFA methyl esters (C4-C10) were isolated from milk fat methyl esters by double vacuum distillation using a 30-cm long Widmer column (Figure 1). A Cartesian manostat was used to control the vacuum. For the first distillation, the pressure was slowly decreased from 140 to 1.0 Torr and the column head temperature was slowly increased from 65°C to 120°C. The pot residue was checked by GC to assure the complete distillation of C4-C10 fatty acid methyl esters. The first distillate was distilled again under reduced pressures,

which was slowly reduced from 140 to 1.0 Torr and the column head temperature was increased from 65°C to 78°C. The fatty acid composition of the second distillate and pot residue was determined by GC to ensure complete distillation of C4-C10 and the inclusion of minimized amount of longer-chain methyl esters in the distillate.

A modification of the saponification procedure described by Markley (17) was used to release the free fatty acids from the short-chain methyl esters. Potassium hydroxide (380 g) was dissolved in 360 ml of water. Short-chain methyl esters (1L) was added with vigorous stirring, followed by 10 ml 100% ethanol. The mixture was heated to boiling under an air condenser and then heating was stopped. Excess methanol and ethanol were removed with a rotary evaporator at 45°C, and then 600 ml concentrated hydrochloric acid (Fisher Scientific, Fair Lawn, NJ) was added while keeping the reaction flask on ice. The upper phase was recovered and dried over anhydrous sodium sulfate. The fatty acid composition of the pooled SCFA mixture was determined by GC.

Short-chain triglyceride preparation. Short-chain triglycerides (SCTG) were synthesized from both commercial fatty acids and those isolated from milk fat using a modification of the method described by Whitehouse (10). For example, 7.26 moles of even numbered C4:0 to C10:0 free fatty acids (Sigma Chemical Co., St. Louis, MO), 21.42 g of p-toluenesulfonic acid (Sigma Chemical Co., St. Louis, MO), 2.305 moles of glycerol (Sigma Chemical Co., St. Louis, MO), and 458 ml toluene (Fisher Scientific, Fair Lawn, NJ) were refluxed in a 5-L round-bottom flask connected to a Dean-Stark water trap for 6 hr. The reaction was considered complete when no more water dripped into the trap.

After the esterification reaction, the mixture was spotted on silica gel thin-layer chromatography (TLC) plate and the TLC plate was developed in hexane:ethyl ether:acetic acid (50:50:1, V/V/V) solution, sprayed with 0.1% dichlorofluorescein in methanol and visualized under ultraviolet light to ensure no mono- and di-glycerides remained in the mixture. The reaction mixture was then washed with 5% sodium carbonate solution and several times with water to neutralize and remove catalyst and excess free fatty acids. The SCTG was dried on a rotary evaporator at 85°C to remove water and toluene. The SCTG synthesized from commercial acids was bleached by stirring with 3 % bleaching earth (AOCS Official Natural Bleaching Earth, Champaign, IL) at 120°C for 5 mins, and filtered through P5 filter paper (Fisher Scientific, Pittsburgh, PA) under vacuum when the oil was still warm (AOCS Official Method Cc 8b-52) (18). The fatty acid compositions of pooled SCTG synthesized from synthetic and natural milk fat sources were determined by GC.

Interesterification of short-chain triglycerides and high-oleic sunflower oil. Short-chain triglycerides from both synthetic and natural sources were interesterified with high-oleic sunflower oil (HOSO) (Trisun 80, RBD, AC Humko, Memphis, TN) at a SCTG : HOSO ratio 1:8.82 in order to produce a fat that had the same percentage of SCFA as that of milk fat. SCTG from the commercial source was also interesterified at a SCTG:HOSO ratio 1:7.19 to produce a fat that had a level of SCFA equals to 120% of that in milk fat. Sodium methoxide (Aldrich Chemical Company, St. Louis, MO) was used as a catalyst at 0.5% of total oil weight. The oil was first heated to 65°C in a 5 L 3-neck round bottom flask fitted with a mechanical Tru-bore glass stirring rod and Teflon paddle. The sodium

methoxide powder was quickly added to the oil, and the flask headspace was flushed with nitrogen gas. The interesterification reaction was continued with vigorous stirring for 6 hr at 65°C. After interesterification, 5% acetic acid (Fisher Scientific, Fair Law, NJ) was added to neutralize the catalyst, and the oil was then washed with distilled water several times and dried on a rotary evaporator for 30 min at 90°C. Fatty acid compositions were determined by GC.

Deodorization of modified oils. A pilot-scale continuous deodorizer similar to the one described by Smouse (19) was used to deodorize the interesterified oils. The oil flow rate was 600 ml/hr, the column temperature 180°C, pressure 0.5 Torr., and the steam rate 12.6 ml/hr. Each batch of deodorized oil was tasted by two experienced observers to ensure that the flavor was good. The deodorized oil was stored at 4°C for cheesemaking. The fatty acid compositions of the deodorized oils were determined by GC.

Determination of residual toluene by direct GC. The residual toluene in modified and deodorized oil was checked by direct injection on a HP 5890 Series II Gas Chromatograph (Heweltt-Packer Company, Avondale, PA) equipped with a flame-ionization detector (FID) (20, 21). About 30 mg oil sample was weighed onto the glasswool packed in the inlet liner. The septum, nut septum and retainer nut of the GC were removed, the liner with sample was inserted into the injection port of the GC, and the inlet system was closed rapidly. The oven was programmed from 40°C for 5 min, to 100°C at a rate of 5°C/min. The injector temperature was 100°C and the detector temperature was 200°C.

Fatty acid analysis by gas chromatography. A modification of the method of Vangtal and Hammond (22) was used to conduct fatty acid analysis of milk fat and modified vegetable oils. A HP 5890 Series II Gas Chromatograph (Heweltt-Packer Company, Avondale, PA) with a fused silica capillary column SPB-1 (30 m x 0.25 mm i.d., 0.25μm) (Supelco, Inc., Bellefonte, PA) was used. The oven temperature was programmed from 140°C for 4 min to 300°C at a rate of 5°C/min and held at 300°C for 6 min. The injector temperature was 280°C and the detector temperature was 300°C. The carrier gas (helium) flow rate was 1.73 ml/min, the split ratio was 4.6. The hydrogen flow rate was 30 ml/min and the air flow rate was 380 ml/min. The fats were converted to their decyl ester derivatives by reacting fat with excess decanol (Aldrich Chemical Company, St. Louis, MO) and 2% sulfuric acid (Fisher Scientific, Fair Lawn, NJ) at 56°C overnight. After the reaction, 1 ml hexane was added and the hexane layer was recovered. The decyl esters were either injected into the GC without further purification or were freed of excess decanol. To separate the excess decanol from the decyl esters, 0.1 ml hexane extract was diluted with 0.5 ml hexane and applied to a 900-mg silica cartridge (Alltech Associates, Inc., Deerfield, IL), which had been preconditioned with 5 ml hexane. Six ml 5% diethyl ether in hexane was used to elute the decyl esters from the cartridge. The solvent was evaporated under nitrogen gas. One ml hexane was added to the sample, and 1 μl sample was injected into the GC. The fatty acid composition of milk fat samples prepared with and without cartridge purification were compared. To quantify individual fatty acids, the fatty acids were divided into three chain length groups, namely, C2-C8, C10-C14, and

C16-C22, and C5:0, C13:0, and C17:0 fatty acids were used as internal standards for each group, respectively. The relative flame ionization detector (FID)-response factors of each individual fatty acid to the internal standards were determined. A recovery study of the individual fatty acids using present GC method and the relative FID-response factors also was conducted.

The fatty acid composition of pooled milk fat methyl ester distillate was determined by direct injection of methyl esters into the same GC column as described above. The oven temperature was programmed from 30°C for 5 min, to 250°C at a rate of 8°C/min and held at 250°C for 5 min. The injector temperature was 220°C, and the detector temperature was 250°C. To determine the fatty acid composition of HOSO, HOSO was converted to methyl esters by using sodium methoxide as a catalyst. The HOSO methyl esters were seperated on a Hewlett-Packard Model 5890 gas chromatograph (Avondale, PA) equipped with a J & W Scientific (Deerfiled, IL) DB-23 fused-silica column (15m, 0.25 mm i.d., 0.25 μm film thickness). The oven temperature was 220°C, the injector temperature was 250°C, and the detector temperature was 250°C (23).

Results and Discussion

The relative FID-response factors of each individual fatty acid decyl ester to the internal standards and the recovery of each fatty acids are presented in Table 1. Relative FID-response factors close to 1.00 for each individual fatty acid decyl esters were obtained by dividing the fatty acids into the three chain-length groups. The recovery of each

individual fatty acid by using the present GC method and the relative FID factors ranged from 93.3% to 107.9%.

The fatty acid composition of the butter fat used in this study is presented in Table 2. Results from two sample preparation methods are listed. One method included a silica cartridge purification step; the other further simplified the method by direct GC injection of the unfractionated transesterification mixture. The results were quite similar. Under the GC conditions optimized in this study, the decanol emerged from the GC column before any of the decyl esters as shown in Figure 2; therefore, the cartridge purification step was not necessary for analysis of milk fat or simulated milk fat. In the case of cheese fat fatty acid analysis, since C2:0 and C3:0 may be present and the decanol may be present in large excess, it will be desirable to remove excess decanol from the decyl esters and concentrate the decyl esters by a cartridge fractionation step before GC injection. The butter fat fatty acid composition determined in this study was similar to the other published data (24) as indicated in Table 2.

To isolate SCFA from milk fat, milk fat was first converted to methyl esters. A double distillation with a Widmer column gave a product with a very small amount of methyl laurate and only traces of methyl myristate in the distillate as shown in Table 3. No C4-C10 methyl esters were found in the pot residue by GC. The distillate composition calculated from the measured milk fat compositions (Table 2) is presented in Table 3. The composition of the methyl ester distillates was close to the expected value. The free fatty acids recovered after saponification and acidification of short-chain methyl esters had

lower C4:0 content than that of the methyl esters (Table 3). This indicated significant loss of C4:0 occurred during the saponification and acidification of methyl esters.

Fatty acid compositions of SCTG synthesized from commercial and milk fat SCFA are presented in Table 4. SCTGs synthesized from both SCFA sources had a fatty acid profile very close to the calculated percentage. SCTG synthesized from milk fat short-chain fatty acid isolates had a C4:0 content lower than that of original milk fat, the loss of C4:0 happened in the saponification and acidification of milk fat methyl esters, and no significant loss of C4:0 occurred in the synthesis of SCTG. Using toluene instead of benzene to remove water from the transesterification system and drive the reaction to the completion seemed to give satisfactory results. The reaction time was shortened to 6 hr instead of 20 hr as reported by Whitehouse (10) using the benzene azeotrope method. The SCTG synthesized from commercial short-chain fatty acids had a darker color than those produced by the benzene azeotrope method. The higher reaction temperature possibly contributed to the darker color. The color of SCTG can be partially removed by bleaching. The SCTG synthesized from milk fat short-chain fatty acids was colorless. The SCTG produced in this manner was quite bitter and disagreeable, in agreement with reports by Johnson (11) and Whitehouse (10). We tried to purify the SCTG by passage through an alumina column (25), followed by deodorization at 80°C for 1.5 hr to remove solvent. This process should remove free fatty acids and partial glycerides, but the bitter, disagreeable taste remained in the SCTG. We concluded that the bitter taste was not due to

mono- and di-glycerides or other polar impurities. To avoid the bitter taste, the SCTG had to be interesterified with the long-chain high-oleic sunflower oil.

The SCTGs were interesterified with HOSO at ratios that would give 100% or 120% level of short-chain fatty acids in milk fat. Whitehouse (10) noticed the bitter off-flavor in the interesterified vegetable oils, and the off-flavor could only be removed through high temperature deodorization at 240°C for one hour. This caused large amount of loss in short-chain fatty acids during deodorization. In this study, we increased sodium methoxide catalyst to 0.5% of oil weight instead of 0.2% as used by Johnson (11) and Whitehouse (10). The off flavor in the interesterified oil was absent after solvent removal by deodorization at 100°C for 1.5 hr. We concluded that 0.5% catalyst gave a more complete interesterification between SCTG and HOSO, so less SCTG was left in the mixture. The fatty acid compositions of washed and dried modified HOSOs are presented in Table 5. The short-chain fatty acids in modified HOSOs with 100% short-chain fatty acids incorporated were very close to the calculated values. The short-chain fatty acids all were between 91.3% and 101.5% of the expected values. The modified HOSO with 120% short-chain fatty acid incorporated had lower short-chain fatty acids than expected, which ranged from 81.9% to 91.1% of the expected values. This indicated a greater loss of SCFA in the washing steps.

The fatty acid compositions of washed, dried, and deodorized modified HOSO are presented in Table 6. All modified HOSOs had certain levels of C4-C10 fatty acids incorporated compared to the original HOSO. No residual toluene was detected by direct

GC method in any of these modified HOSOs after deodorization. All deodorized oils had plain acceptable flavor as judged by two experienced observers. About 2% oil was lost during deodorization and this loss might explain the lower short-chain fatty acid contents in the deodorized oils (Table 6) compared to that of the undeodorized oils (Table 5).

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Table 1. Relative FID-response factors of individual fatty acid decyl esters and the recovery of individual fatty acids

Free Fatty Acids	FID-Response Factors[a]	Recovery (%)[b]
C 2:0	1.010 ± 0.002	105.9 ± 6.9
C 3:0	1.006 ± 0.001	107.9 ± 5.3
C 4:0	0.973 ± 0.000	100.8 ± 1.5
C 6:0	0.989 ± 0.000	97.2 ± 6.0
C 8:0	1.016 ± 0.003	102.1 ± 5.1
C 10:0	0.983 ± 0.002	99.2 ± 8.1
C 12:0	0.996 ± 0.000	104.1 ± 2.3
C 14:0	1.016 ± 0.001	103.7 ± 2.8
C 16:0	0.909 ± 0.000	93.3 ± 1.6
C 18:0	0.987 ± 0.000	101.7 ± 0.9
C 20:0	1.061 ± 0.001	107.0 ± 2.3
C 22:0	1.142 ± 0.002	95.0 ± 6.1

[a]Means of four replications.
[b]Means of seven replications.

Table 2. Fatty acid compositions of butter fat determined with or without purification of decyl esters by a silica cartridge[a]

Free Fatty Acids	With Cartridge Purification (%)	Without Cartridge Purification (%)	Butter Fat[b] (%)
C 4:0	3.69 ± 0.15	3.60 ± 0.54	3.4
C 6:0	2.19 ± 0.07	2.12 ± 0.30	2.1
C 8:0	1.23 ± 0.03	1.20 ± 0.15	1.2
C 10:0	2.63 ± 0.05	2.61 ± 0.24	2.6
C 12:0	3.01 ± 0.04	3.04 ± 0.20	3.0
C 14:0	10.78 ± 0.46	10.87 ± 0.67	10.6
C 16:0	30.54 ± 0.51	30.50 ± 0.45	27.7
C 18:0	14.81 ± 0.77	14.60 ± 0.01	12.8
Pooled C18:1, C18:2, C18:3	30.66 ± 0.35	30.95 ± 0.38	30.5
C 20:0	0.24 ± 0.04	0.20 ± 0.02	
C 20:1	0.16 ± 0.02	0.21 ± 0.02	
C 22:0	0.06 ± 0.01	0.05 ± 0.01	

[a]Means of five replications.
[b]From German et al. (24).

Table 3. Fatty acid compositions of pooled milk fat methyl ester distillates and pooled short-chain fatty acids mixture after saponification and acidification of milk fat fatty acid methyl esters

Free Fatty Acids	Pooled Milk Fat Methyl Ester Distillates (%)[a]	Pooled Short Chain Fatty Acids Mixture (%)[a]	Percentage Expected (%)[b]
C 4:0	35.87 ± 0.42	27.45 ± 0.36	38.14
C 6:0	24.26 ± 0.08	23.82 ± 0.10	22.68
C 8:0	13.20 ± 0.07	14.33 ± 0.19	12.37
C 10:0	22.09 ± 0.33	26.30 ± 0.10	26.80
C 12:0	4.22 ± 0.39	6.81 ± 0.01	0.00
C 14:0	0.40 ± 0.08	1.30 ± 0.01	0.00

[a]Means of three replications.
[b]Calculated according to the butter fat fatty acid composition reported in Table 2.

Table 4. Fatty acid compositions of short-chain triglycerides (SCTG) synthesized from synthetic and natural milk fat short-chain fatty acids

Free Fatty Acids	Percentage in SCTG Synthesized from Synthetic Source (%)[a]	Percentage Expected (%)[b]	Percentage in SCTG from Milk Fat Source (%)[a]	Percentage Expected (%)[b]
C 4:0	36.17 ± 0.14	38.14	25.55 ± 0.18	27.45
C 6:0	21.98 ± 0.13	22.68	23.29 ± 0.16	23.82
C 8:0	12.95 ± 0.19	12.37	15.33 ± 0.18	14.33
C 10:0	28.90 ± 0.43	26.80	27.43 ± 0.45	26.30
C 12:0	0.00 ± 0.00	0.00	7.07 ± 0.02	6.81
C 14:0	0.00 ± 0.00	0.00	1.33 ± 0.02	1.30

[a]Means of three replications.
[b]From Table 3.

Table 5. Fatty acid compositions of modified high-oleic sunflower oils (HOSO) before deodorization

Free Fatty Acids	HOSO with 100% commercial SCFAs (%)[a]	Expected values of SCFAs (%)[b]	HOSO with 120% commercial SCFAs (%)[a]	Expected Values of SCFAs (%)[b]	HOSO with 100% milk SCFAs (%)[a]	Expected values of SCFAs (%)[b]
C 4:0	3.36 ± 0.03	3.68	3.62 ± 0.02	4.42	2.48 ± 0.06	2.60
C 6:0	2.16 ± 0.01	2.24	2.34 ± 0.02	2.69	2.26 ± 0.06	2.37
C 8:0	1.34 ± 0.02	1.32	1.44 ± 0.01	1.58	1.46 ± 0.04	1.56
C 10:0	2.85 ± 0.04	2.94	3.21 ± 0.23	3.53	2.70 ± 0.02	2.79
C 12:0	0.00 ± 0.00		0.00 ± 0.00		0.69 ± 0.00	
C 14:0	0.00 ± 0.00		0.00 ± 0.00		0.17 ± 0.01	
C 16:0	2.98 ± 0.02		3.53 ± 0.01		3.56 ± 0.07	
C 18:0	3.76 ± 0.01		3.24 ± 0.09		3.25 ± 0.02	
Pooled C18:1-18:3	81.90 ± 0.05		80.91 ± 0.14		81.55 ± 0.24	
C 20:0	0.38 ± 0.01		0.43 ± 0.04		0.51 ± 0.06	
C 20:1	0.23 ± 0.02		0.34 ± 0.02		0.36 ± 0.01	
C 22:0	1.04 ± 0.03		0.95 ± 0.07		1.01 ± 0.02	

[a]Means of four replications

[b]Calculated according to the true SCTG fatty acid compositions (Table 4) and the SCTG:HOSO ratios used in the interesterification.

Table 6. Fatty acid compositions of modified high-oleic sunflower oil (HOSO) after deodorization[a]

Free Fatty Acids	HOSO with 100% synthetic SCFAs (%)	HOSO with 120% synthetic SCFAs (%)	HOSO with 100% milk SCFAs (%)	HOSO[b]
C 4:0	2.71 ± 0.17	2.81 ± 0.02	2.37 ± 0.01	0.00
C 6:0	1.78 ± 0.12	2.15 ± 0.02	2.19 ± 0.01	0.00
C 8:0	1.05 ± 0.05	1.34 ± 0.01	1.48 ± 0.02	0.00
C 10:0	2.04 ± 0.17	2.67 ± 0.02	2.25 ± 0.02	0.00
C 12:0	0.00 ± 0.00	0.00 ± 0.00	0.66 ± 0.01	0.00
C 14:0	0.00 ± 0.00	0.00 ± 0.00	0.17 ± 0.00	0.05
C 16:0	3.29 ± 0.01	2.98 ± 0.01	3.46 ± 0.01	3.44
C 18:0	3.63 ± 0.12	3.13 ± 0.12	3.40 ± 0.03	4.01
Pooled C18:1-18:3	83.22 ± 0.60	82.95 ± 0.11	81.84 ± 0.18	90.42
C 20:0	0.39 ± 0.00	0.33 ± 0.00	0.41 ± 0.00	0.39
C 20:1	0.30 ± 0.01	0.32 ± 0.00	0.33 ± 0.00	0.31
C 22:0	1.12 ± 0.01	1.08 ± 0.00	1.15 ± 0.00	1.00
C 24:0	0.37 ± 0.02	0.33 ± 0.00	0.36 ± 0.00	0.31

[a]Means of four replications.
[b]Determined as methyl esters.

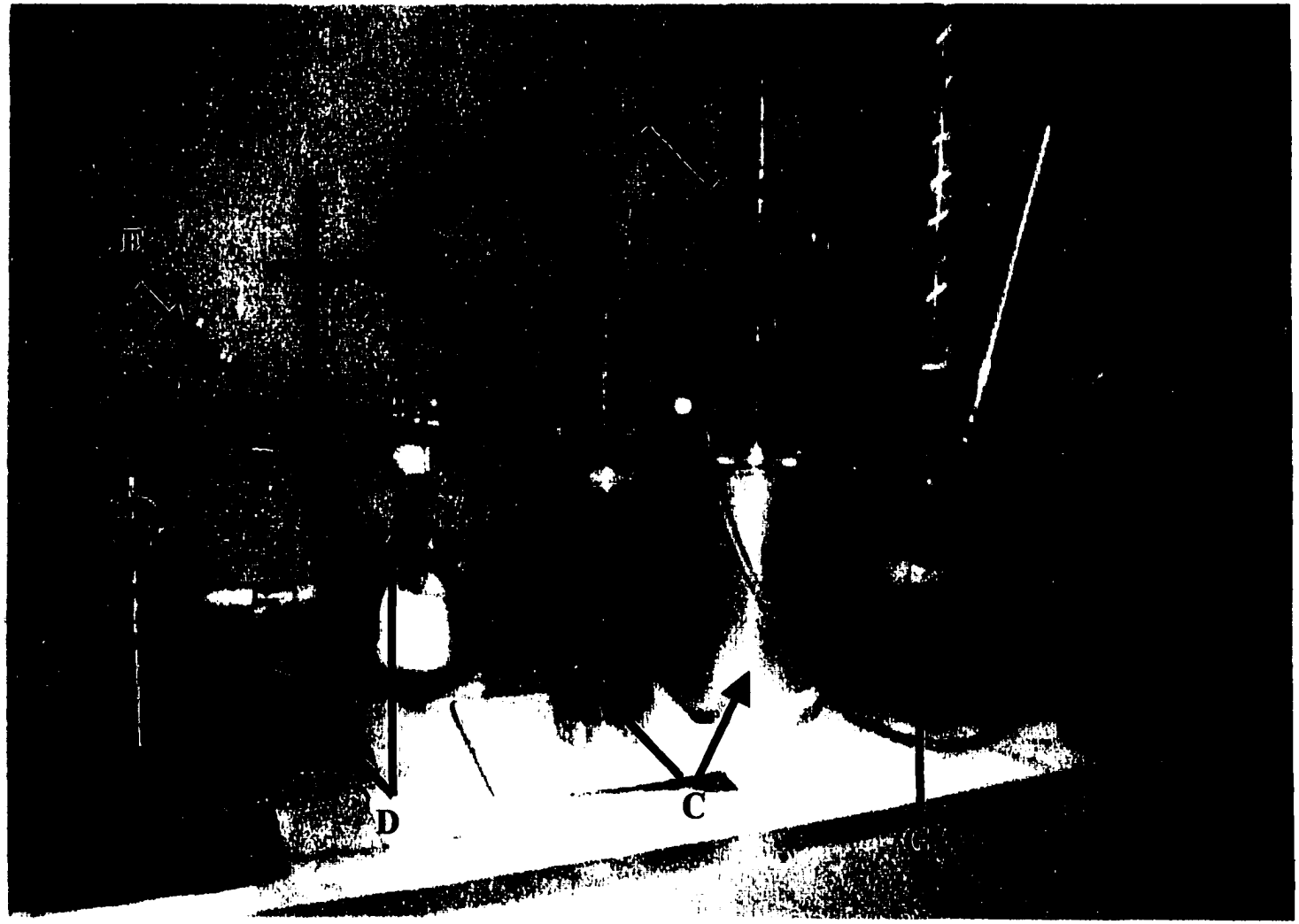

Figure 1. Milk fat fatty acid methyl esters distillation apparatus (A:Widmer column; B: Condenser; C: Cold traps; D: Manometers; E: Air leak; F: Manostat; G: Magnetic stirrer; H: Voltage control)

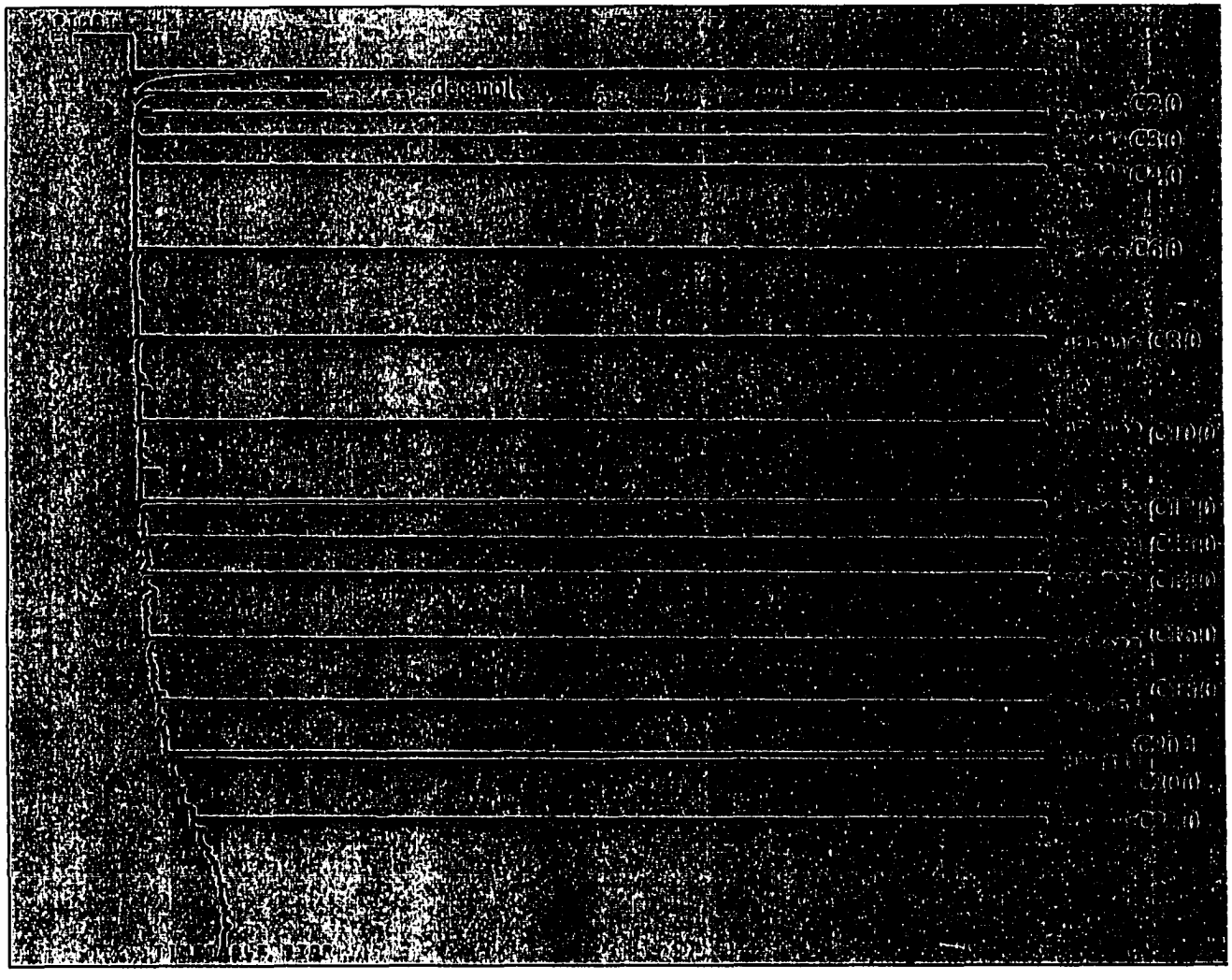

Figure 2. GC chromagram of C2-C22 decyl ester standards after purification with a silica cartridge

CHAPTER 3. PRODUCTION AND CHARACTERIZATION OF SWISS CHEESE-LIKE PRODUCT FROM MODIFIED VEGETABLE OILS

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Abstract

The objectives of this study were to produce high quality Swiss cheese from modified vegetable oils and skim milk and to determine the sensory, chemical, and physical properties of the cheeses. Seven types of Iowa-style Swiss cheeses were produced by recombining skim milk with various fat sources, namely, high-oleic sunflower oil (HOSO); milk fat; randomized milk fat; HOSO with commercial short-chain fatty acids (SCFA) interesterified at 100% and 120% of the levels in the milk fat; HOSO with milk fat SCFA interesterified; and HOSO with free fatty acids (C4-C10) dissolved. The sensory, chemical, and physical analyses were conducted to evaluate the flavor and texture of the cheeses.

No differences were found in bitterness and caramelized flavor among treatments. All cheeses made from modified HOSO had significantly higher scores in typical Swiss flavor and volatiles than unmodified HOSO and were not significantly different from milk fat controls. Simple correlation of sensory flavor and chemical parameters indicated that Swiss flavor was positively correlated with sweetness, volatiles, caramelized flavor, non-fat solids, C4-C10 fatty acids, and negatively correlated with fat and salt content. A linear regression model was established for typical Swiss flavor which included fat, salt, titritable acidity, and C12-C16 fatty acids as variables ($R^2 = 0.91$). Instrumental texture profile

analysis indicated no differences among the treatments in texture attributes except for cohesiveness; however, sensory panelists detected differences among the treatments in hardness and springiness. The commercial feasibility of the production of Swiss cheese from modified HOSO was discussed.

Key words: Swiss cheese, vegetable oil, high-oleic sunflower oil, cheese flavor, cheese texture, filled cheese, low-fat cheese.

Introduction

Milk fat has been considered hypercholesterolemic because it contains cholesterol and a great proportion of saturated fatty acids (1). The consumer's demand for healthy and nutritionally balanced products has led to the development of a number of fat-free and low-fat cheese products. However, the flavor, texture, and shelf life of these cheeses has been described as either "lacking", "changing", or "improving with further research" (2, 3, 4, 5). Consumers and processors are still seeking a more desirable nutritional cheese products. Vegetable oils have long been used to substitute for butterfat in the manufacture of cheeses (6, 7). There are some advantages of using vegetable oils in place of butterfat in cheesemaking. The vegetable oils are cholesterol-free, cheaper than milk fat, and some are more stable and more easily stored. Vegetable oils often have uniform quality without seasonal change and security of supply (8, 9). However, the vegetable oil cheeses were reported to have an oily off-flavor, less volatile fatty acids (10), lower sensory scores than normal milk fat cheese, and a soft and crumbly texture (8, 11, 12, 13). Whitehouse (14)

and Johnson (15) made Swiss cheese by using vegetable oils that had been modified by interesterifying short-chain fatty acids. Their results showed great promise of making quality Swiss cheese with modified vegetable oils.

At Iowa State University, HOSO has been successfully modified to resemble milk fat by incorporating SCFA (C4-C10). The modified HOSO had a SCFA composition closely resemble that of milk fat (16). In this study, Iowa-style Swiss cheeses were produced from modified HOSO and skim milk. The sensory, chemical, and physical characteristics of cheeses produced from modified vegetable oils were compared with those of controls made from HOSO, milk fat, and randomized milk fat emulsified into the skim milk under similar homogenization conditions. Furthermore, the possibility of making cheese from skim milk and HOSO with certain amounts of free SCFA (C4-C10) simply dissolved in the HOSO was explored. Therefore, the objectives of this study were to find the optimal processing conditions for producing high quality Swiss cheese from modified HOSO and skim milk and to determine the sensory, chemical, and physical properties of cheeses. Seven types of Swiss cheeses from different fat sources were made in duplicate.

Experimental Procedures

Preparation of fat sources. High-oleic sunflower oil (Trisun 80, RBD) was purchased from AC Humko (Memphis, TN). Seven types of fat were prepared in 6 kg amounts. Milk fat was isolated from USDA Grade AA sweet butter (Prize of Iowa, Mid-

America Farms, Springfield, MO) according to the method described by Yu and Hammond (16).

HOSO with commercial SCFA (C4-C10) incorporated at 100% and 120% of the levels in the milk fat, and HOSO with SCFA from milk fat incorporated were available from a previous study (16).

HOSO with C4-C10 commercial SCFA (Sigma Chemical Co., St. Louis, MO) dissolved was prepared on the day when cheese was made by adding C4-C10 free fatty acids at the amounts that were normally found in the typical Swiss cheeses as determined by Vangtal (17). In each 2.8 kg HOSO, 3.66 g of C4:0, 0.57 g of C6:0, 0.57 g of C8:0, and 1.13 g of C10:0 were added. The amount of C4:0 used included 7% possible loss of C4:0 during the cheesemaking procedure because of its solubility in whey as determined in a preliminary study.

Randomized milk fat was prepared according to the methods described by Kuksis et al. (18) and Kuksis et al. (19). Milk fat was first dried by using Drierite (W.A. Hammond Drierite Company Ltd., Xenia, OH) at a Drierite:oil ratio 1:5 and shaking on a mechanical shaker for 3 hrs at 45°C (20). The fat was filtered through a P5 filter paper (Fisher Scientific, Pittsburgh, PA) under vacuum while placing an infrared lamp on the top of the filtration system to keep the fat from solidifying. Dried milk fat in 500 g batches was heated to 100°C with continuose stirring, sodium methoxide was added at 0.5% of oil weight, the flask headspace was flushed with nitrogen gas, and the rearrangement was continued for 2 hr at 100°C with vigorous stirring. At the end of the reaction, 15 ml 20% citric acid was added and the fat was stirred for additional 5 min. The fat was washed with

hot water several times and dried on a rotary evaporator for 40 min at 90°C. The randomized milk fat was deodorized at 200°C for 1.5 hr using a lab scale steam deodorizer (21).

Preparation of cultures used for cheesemaking. *Lactobacillus 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 10% reconstituted non-fat dry milk (Carnation Co., Los Angeles, CA) for 48 hr with 1% inoculation and incubated at 40°C and 37°C, respectively. *P. shemanii* P19 was inoculated at 2% in sodium lactate broth and incubated at 32°C for 48 hr. Two more transfers were made to obtain 18-hr active cultures. All cultures were mixed with pure glycerol at a 2:1 ratio, and distributed aseptically into individual, autoclaved 1.7-ml plastic centrifuge tubes at 1.0 ml for each tube. Cultures were stored at -70°C. When a culture was needed, it was thawed, inoculated at 1% into the appropriate medium, and incubated for 36 hr. Two additional transfers of 18-hr cultures were made to obtain active cultures for cheesemaking. A commercial mesophilic DVS Freeze-dried culture R-704 was purchased from Chr. Hansen's Inc. (Milwaukee, WI). The culture was distributed aseptically into individual 14-ml sterile plastic tubes at 2 g cultures per tube. Each tube contained approximately 15 units culture and would inoculate from 68 kg-136 kg milk. The cultures were stored at – 40°C and added directly to the milk during cheesemaking.

Homogenization of fat sources into skim milk. Skim milk (Anderson & Eridson, Des Moines, IA) was purchased from a local food store. A Gaulin two-stage homogenizer

(Model 18M-8TA, AVI Gaulin Corp., Wilmington, DE) was used to homogenize the skim milk with various fat sources. The first stage pressure of homogenizer was set at 140 kg/cm^2, and the second stage pressure was set at 50 kg/cm^2. The oil was warmed to 60°C in an oven. Gum acacia (TIC Gums, Belcamp, MD), which was used as an emulsifier, was stirred into the oil at 1.5 % of the oil weight. The skim milk was heated to 60°C in an electric heated kettle (Model TDC/TA/40, Groen Company, Elk Grove, IL). Half of the skim milk (50 kg) was pumped continuously from the kettle to the homogenizer using a peristaltic pump (Masterflex I/P, Cole-Parmer Instrument Company, Vernan Hills, IL) fitted with a Model 7529-10 Easy-Load pump head and #6419-73 Masterflex Tygon food grade tubing at 0.84 L/min flow rate. At the same time, the oil was pumped continuously to the homogenizer using another peristaltic pump (Masterflex Model 7520-00, Cole-Parmer Instrument Company, Vernan Hills, IL) fitted with a Model 7018-52 pump head and #6419-17 Masterflex Tygon food grade tubing at a flow rate of 0.047 kg/min. The milk and oil in the feeding tank were mixed vigorously at 300-rpm by a mixer head (Model 745-5010, Barnant Co., Burlington, IL), which was attached on the top of the homogenizer feeding tank. The homogenized milk was collected in a sanitized milk can and transferred to the cheese vat. The remaining half of the skim milk was added directly into the vat and mixed with the homogenized milk by a mechanical stirrer.

Cheesemaking. Seven types of Iowa-style Swiss cheese were made in duplicate in a completely randomized order. The cheesemaking procedure was followed by the method described by Reinbold (22) (Table 1).

Packaging of cheese. After brine salting, the cheese block was allowed to dry at 4°C. Then the block was vacuum-packed using a Koch vacuum package machine (Koch Supplies, Inc., Kansas City, MO) in a Curlon grade 861 polyethylene pouch (Curwood, Oshkosh, WI). After 3 months ripening, the block of cheese was cut into 20 1-lb portions by using a pneumatic cutting machine equipped with stainless steel cutting wires. The 1-lb blocks were repacked in a 8 x 12 Curlon grade 861 polyethylene pouch and stored at -20°C for later analysis except for the cheeses that were used for texture profile analysis.

Texture profile analysis (TPA). A TA.XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) with Texture Expert for Windows (Version 1.0 Software) installed was used for texture profile analysis (23). The analysis was conducted at the day when each block of cheese was cut and repacked; so, freshly ripened cheese samples were used. Cheese samples were taken from the portions without eyes and at least 1-cm from the edges of the 1-lb block. The samples were cut into cylindrical samples (25-mm diameter x 23-mm height) at 4°C, sealed in an airtight Ziploc bag, and placed in a 23°C incubator for 2 hrs before the analysis (24, 25).

A two-cycle compression test was performed. The machine was calibrated with a 5-kg loading cell. Each sample was compressed to 80% of its initial height by using a 37-mm diameter flat plate probe that is attached to the moving crosshead. The crosshead speed was 18 mm/min (25, 26). The test was conducted at room temperature. Ten samples were tested for each treatment. The texture parameters (hardness, adhesiveness, springiness, cohesiveness, gumminess, and chewiness) were calculated from the textural profile curves by Texture Expert Software (Figure 1). Hardness was defined as the

maximum force (N) recorded during the first compression cycle; adhesiveness was the negative force area for the first cycle, representing the work necessary to pull the compressing plunger away from the sample; springiness was the ratio of the width of the down stroke of second cycle to the width of the down stroke of first cycle; cohesiveness was the ratio of the positive area during the second compression to that during the first compression; gumminess was the product of hardness x cohesiveness; and chewiness was the product of gumminess x springiness.

Sensory analysis of cheese. The sensory analysis of cheese was conducted in a completely randomized experimental design. Both flavor and texture attributes of Swiss cheese were evaluated. Fourteen panelists were selected from faculty, staff, and students in the Department of Food Science and Human Nutrition at Iowa State University. All panelists were required to sign an Iowa State University Human Subject Informed Consent Form. Panelists were trained in four 1-hr sessions in an attempt to reach an agreement on the terminology and intensity of each sensory attribute. During the training sessions, panelists were instructed about how to use the score card, familiarized with the flavor and texture attributes of Swiss cheese, and instructed in special tasting techniques used in flavor and texture evaluations. The training samples included some commercial cheeses and Iowa-style Swiss cheeses that were produced at Iowa State University. The samples represented a wide range of all flavor and texture attributes of Swiss cheese.

A 15-cm line scale was used for both flavor and texture evaluation. For the flavor test, sweetness, saltiness, sourness, bitterness, volatiles, typical Swiss cheese flavor, and caramelized flavor were evaluated (Appendix A). The absence of sweetness, saltiness,

sourness, bitterness, volatiles, typical Swiss cheese, and caramelized flavor was scored 0; strong sweetness, saltiness, sourness, bitterness, volatiles, typical Swiss cheese flavor, and caramelized flavor was scored 15. For texture analysis, hardness, cohesiveness, and springiness were evaluated (Appendix B). To determine the hardness, panelist were advised to place the sample between their molars, bite through it once and evaluate the force required to bite into the cheese. Cohesiveness was evaluated by placing the sample between their molars, compressing it fully and noting the degree to which it deformed rather than broke. For springiness, the panelists were advised to place the sample between their molars, compress it partially without breaking the structure, release the tooth pressure and evaluate the degree to which the cheese returned to its original shape. The zero end of the texture scales were labeled soft for hardness, breaks for cohesiveness, and not springy for springiness, respectively; the high end of the scales were labeled hard, deforms, and very springy and were scored 15.

To prepare the samples, cheeses were thawed overnight at 4°C. The samples were taken at least 2 cm away from the edges of 1-lb block and cut into 1-cm cubes. The samples were tempered in airtight Ziploc bags for half an hour at room temperature. Six randomly selected cheese cubes of each treatment were placed into a 3-oz paper cup coded with a random 3-digit number. Four samples were evaluated in each session. Two sessions were held each day with the flavor session in the morning and the texture session in the afternoon. The samples were presented to panelists in a randomized order. Panelists evaluated samples in individual booths and were instructed to rinse their months with distilled water and to eat unsalted crackers between samples.

Sample preparation for free fatty acids and other chemical analysis. Samples for free fatty acids and chemical analysis were prepared according to the AOAC Official Methods 955.30 (27). Cheeses were thawed at 4°C overnight. The center portion of cheese block was cut into strips and grated with a cheese grater.

Free fatty acids analysis. Free fatty acids were extracted from cheese matrix by the method described by de Jong and Badings (28). Duplicate 1.0-g of the grated cheese sample was ground with 3.0-g anhydrous sodium sulfate (Fisher Scientific, Fair Lawn, NJ) using a mortar and pestle. The sample was transferred to a 5-dram vial and 0.3 ml 2.4 M sulfuric acid and 1.0 ml internal solution containing C5:0, C13:0, and C17:0 free fatty acids (Sigma Chemical Co., St. Louis, MO) were added. The mixture was extracted three times with 3 ml ethyl ether:heptane (1:1, V/V) solution, and the extract was clarified by centrifugation at 300 rpm for 2.5 mins. The upper layers were combined and dried with 1.0-g anhydrous sodium sulfate. The combined solvent extracts were applied on a 500-mg Extract Clean NH_2 cartridge (Alltech Association, Inc., Deerfield, IL), which was preconditioned with 10 ml heptane (Fisher Scientific, Fair Lawn, NJ). The neutral lipids were eluted from the column with 4 ml chloroform:2-propanol (2:1, V/V). Free fatty acids were eluted with three 2-ml lots of 2% formic acid (Sigma Chemical Co., St. Louis, MO) in diethyl ether, and the elutes were placed in a 5-dram vial. The free fatty acids were converted to their sodium salts by adding 1.5 ml 10% sodium carbonate solution and shaking vigorously for 5 min. Diethyl ether was evaporated under nitrogen, and water was removed by placing the vial on a steam bath (29). After the water was completely removed, 300 µl decanol and 100 µl concentrated sulfuric acid were added and the vial

was shaken vigorously to allow all salts to dissolve, sealed, and placed in a 56°C oven overnight. After the esterification reaction, 0.5 ml water and 0.5 ml hexane was added and the vial was centrifuged at 3000-rpm for 2 min. The upper hexane phase was applied onto a 900-mg Maxi-clean silica cartridge (Alltech Associate, Inc., Deerfield, IL), which was preconditioned with 5 ml hexane. The decyl esters were eluted from the column with 6 ml 5% ether in hexane solution. The solvent was evaporated under nitrogen and the residue in the vial was redissolved in 0.5 ml hexane. One μl of the solution was injected into the gas chromatograph (GC). The GC conditions were the same as those described by Yu and Hammond (16).

Chemical analysis. The moisture, fat, salt, pH, and titratable acidity (TA) of the cheeses were determined in duplicate for all treatments. Moisture was determined according to the AOAC Official Method 926.08. Fat in cheese was determined according to the AOAC Official Method 933.05. Salt was determined by using a DiCromet Salt Analyzer (Model DSA-1000, Diamond Crystal Salt Company, St. Clair, MI). Ten grams of prepared cheese sample was weighed and blended with 100-ml distilled water in a food blender. The slurry was filtered through a drip coffee maker filter paper. The salt in the filtrate was measured with the salt analyzer using 0.15% sodium chloride solution to standardize the instrument to read 1.5. The salt percentage of the sample was then read directly from the digital display of the machine. The pH of the filtrate used for salt determination also was measured by a pH meter (Orion Model SA 720, Orion Research Inc., Beverly, MA). The pH meter was calibrated with pH 7.0 and pH 4.0 buffers. The titratable acidity of the cheese was determined according to the AOAC Official Method

920.124 by titrating 25 g of the cheese filtrate prepared for salt determination with 0.1 N NaOH using phenolphthalein as an indicator. The results were expressed as % lactic acid.

Statistical analysis. Analysis of variance (ANOVA) (30) was used to analyze the sensory, chemical, and physical analysis data. When F-values were significant, least significant differences (LSD) at $p \leq 0.05$ were calculated. Principal component analysis was performed for fatty acid data using SAS FACTOR procedures. Correlation analysis was performed to correlate sensory results with those of instrumental analysis using the SAS CORR procedure. Multiple linear regression was performed using the SAS REG procedure to generate a model for volatile and typical Swiss flavor.

Results and Discussion

Flavor characteristics of Swiss cheese. The results of sensory flavor profiles and the free fatty acids analyses are presented in Table 2 and 3. Among all the flavor attributes, bitterness and caramelized flavor were not significantly different among the treatments. This finding agreed with Johnson (15) who found no differences among treatments in bitterness and burned flavor when Swiss cheeses were made from corn oil and corn oil containing SCFA. All cheeses made from chemically modified HOSOs had significantly higher sensory scores in volatiles and typical Swiss cheese flavor than the cheeses that were made from HOSO or HOSO with SCFA dissolved in the oil. The cheeses made with modified HOSO were not significantly different from the control cheeses that were made from milk fat or randomized milk fat. It seemed that modification

of HOSO by interesterifying SCFA into it really helped to improve the flavor of the cheese made from it.

No major flavor differences were found between milk fat and randomized milk fat cheeses except that randomized milk fat cheese was rated more salty and sour. The more randomized distribution of short-chain fatty acids in randomized milk fat did not cause significant change in cheese flavor. Consequently, we would not expect the randomized distribution of short-chain fatty acids in the modified HOSO to result in inferior cheese flavor. Weihe (21) indicated that randomized and deodorized milk fat recombined into milk scored as well as many market milks. But cakes made with randomized and deodorized butter oil were inferior in flavor and texture to those made with untreated butter oil.

Cheeses that were made from modified HOSO with 100% commercial or milk fat short-chain fatty acids incorporated even had slightly higher scores in typical Swiss cheese flavor and higher C4-C10 free acids than the milk fat controls. Whitehouse (14) and Johnson (15) both found that cheeses made from natural milk fat emulsion had more typical Swiss flavor than the recombined and homogenized milk fat control cheeses. In this study, natural milk emulsion cheese was not made; therefore, a direct comparison between the flavor of the modified vegetable oil cheeses and natural milk emulsion cheese could not be made. But we might still expect that cheese made from natural milk emulsion has the most typical Swiss flavor because milk fat globule membrane plays an important role in cheese flavor development (14, 15). The cheese that was made from modified HOSO with SCFA incorporated at 120% that of milk fat scored the highest in both

volatiles and sourness. The greater C4-C10 free fatty acids in this type of cheese might contribute to the greater scores in volatiles and sourness.

Cheeses made from unmodified HOSOs had the lowest scores in typical Swiss flavor and volatiles. The SCFA (C4-C10) in the unmodified HOSO cheeses were the lowest observed, and almost none were presented. We might therefore expect a correlation between a good flavored Swiss cheese and the presence of SCFA (C4-C10).

There were no significant differences in any flavor attribute among the three types of HOSO cheeses made with interesterified SCFA except in sweetness. Cheeses made from modified HOSO with 100% SCFA incorporated had the highest sweetness scores, while cheese made from HOSO with 120% SCFA incorporated had significantly lower sweetness scores. The lower scores in sweetness and higher scores in sourness and volatiles in the latter cheese might account for its slightly lower Swiss flavor score compared to the other two interesterified HOSO cheeses. Sweetness, sourness, and volatiles were all important flavor characteristics of a typical Swiss cheese (31), but a good flavored Swiss cheese may depend on a well-balanced flavor of all these flavor characteristics.

The acetic and propionic acid contents in the cheeses were all within a normal range (Table 3) (32, 33), which indicated a normal fermentation of the propionic acid bacteria (31). The lack of SCFA (C4-C10) in the cheese produced from HOSO with SCFA dissolved was unexpected, and this might explain the poor flavor scores of this type of cheese. The reason for the loss of SCFA was not clear, but these results suggested that the

added SCFA were metabolized during the cheese ripening and must be continuously replaced by hydrolysis of the cheese triglyceride.

Chemical characteristics of cheese. The results from chemical analysis of cheeses other than for fatty acids are presented in Table 4. The moisture contents in the cheeses ranged from 38.27% to 40.97%, which was very close to the 39% to 41% moisture range of normal Iowa-style Swiss cheese (22).

The fat contents in the cheeses ranged from 24.97% to 30.73% of total cheese weight. Generally, modified vegetable oil cheeses had slightly lower fat contents than did the unmodified vegetable oil cheeses and milk fat control cheeses, but this did not seem to cause any flavor or texture defects in these cheeses. Whitehouse (14) also reported that one set of control cheeses with extremely low fat content had very high sensory evaluation scores. The result suggests that a good quality cheese with reduced or low-fat content could be made as long as a minimal amount of fat was present. Davide (34) indicated that Cheddar cheeses with 25%-reduced fat were as good as full fat ones. The 33% reduced-fat Cheddar cheese was still acceptable, but 50%-reduced fat was not acceptable. Bryant (4) indicated that at fat content below 13% it would be impossible to make a good quality Cheddar cheese.

The salt, pH, and titratable acidity of all cheeses were similar to those of other studies (15, 33). The non-fat solids contents of all treatments were calculated and reported in Table 4. The non-fat solids mainly were protein, amino acids, salts, carbonyl, and minerals. This parameter was negatively correlated with the fat content.

Principal component analysis. To reduce the number of variables and to identify groups of observed variables that tend to hang together empirically, free fatty acids in whole cheese were subjected to a principal component analysis (35). Table 5 shows the factors produced by principal component analysis of fatty acids in whole cheese. The values in the table were the factor loading indicating the contribution of each fatty acids to the factors. The first three components were retained by Eigenvalue-one criteria, i.e. only the components that had a Eigenvalue larger than one were retained. The three retained factors accounted for 83% of the total variance. Factor 1 was composed mainly with C4-C10 SCFA, which were the group of fatty acids used to modify the vegetable oils. The C12-C16 fatty acids were grouped into Factor 2. The first two members of this group are only present in the milk fat. Factor 3 consisted mainly of C2 and C3, which were produced by *Propionibacteria* fermentation. Johnson (15) generated similar factor groups to those in this study except that she combined Factor 1 and 2 into a single factor. The three fatty acid factors instead of 12 individual fatty acids were used as 3 independent variables in the subsequent correlation and linear regression analysis. Factor scores of each factor, which were a linear composite of the optimally weighted observed variables, were calculated from the SAS program (30)

Correlation analysis. A simple correlation was conducted within sensory flavor parameters, within chemical parameters, and between sensory parameters and chemical parameters. Three fatty acids factors that were derived from principal component analysis were included in the chemical parameters. Table 6 shows the correlation of the sensory flavor parameters. Sensory sweetness, volatiles, Swiss flavor, and caramelized flavor were

all correlated. This correlation was not surprising since these flavor notes were all considered good flavors that should be presented in a typical Swiss cheese. Saltiness, sourness, and volatiles were correlated to each other. Bitterness, which was considered as a flavor defect in Swiss cheese (36), was not correlated to any other flavor parameters.

Table 7 shows the correlation of all chemical parameters. Moisture was positively correlated to salt content and titratible acidity and negatively correlated to non-fat solids. The higher moisture content might favor salt retention and provide more lactose for acid production. Fat content was negatively correlated to non-fat solids and Factor 1. Similar results were reported by Johnson (15). Salt content was related to titratable acidity and Factor 1. Non-fat solids was correlated to Factor 1. The correlations among Factor 1 through 3 were zero as expected since these factors were all independent components (35).

The correlation between sensory and chemical parameters are presented in Table 8. Sensory sweetness, saltiness, sourness, volatiles, Swiss flavor, and caramelized flavor were all negatively correlated with the fat content. Sweetness, volatiles, Swiss flavor, and caramelized flavor were all correlated to non-fat solids content. Factor 1 (C4-C10 SCFA), which was the fatty acids we incorporated into the HOSO, significantly contributed to the sourness, volatiles, Swiss flavor, and caramelized flavor. Factor 2 (C12-C16 fatty acids) was not correlated to any other parameters. The replacement of this group of fatty acids with long-chain fatty acids might not cause any changes in cheese flavor. Factor 3 (C2-C3 fatty acids) had a weak correlation with bitterness. Vangtal (17) reported a correlation of proteolysis and bitter flavor; however, proteolysis was not determined in this study.

Multiple linear regression. Sensory volatiles and typical Swiss flavor were regressed on all chemical parameters including the three fatty acids factors. A stepwise procedure was used as the model-selection method to pick up any variables that were significant at the 0.150 level (37). The linear model generated for volatile and typical Swiss flavor were as follows:

Volatile = 6.90 + 1.04 (Factor 1)

Typical Swiss Flavor = 34.27 – 0.64 (% Fat) – 8.72 (% Salt) + 7.96 (%TA) + 0.40 (Factor 2)

The Model for volatiles had an R-square equaled to 0.57, and the model for Swiss flavor had an R-square equaled to 0.91. R-square represents the fraction of variance that is accounted for by the linear combination of predictor variables. Using the variables in equation for Swiss cheese flavor, we could be able to predict the Swiss flavor in the cheeses.

Texture characteristics of cheese. The texture profile analysis of the experimental Swiss cheese is presented in Table 9. There were no significant differences among treatments in hardness, adehesiveness, springiness, gumminess, and chewiness. The only difference noted was in cohesiveness. Two types of milk fat cheeses were significantly less cohesive than the HOSO control cheese. The cohesiveness value of all modified HOSO cheeses fell in between those of HOSO control and milk fat control cheeses, with HOSO cheese being the most cohesive one and the randomized milk fat cheese being the least cohesive one. Similar trend was noticed from the sensory cohesiveness scores (Table

10) except that the milk fat control cheese had higher cohesiveness score than the randomized milk fat cheese, but the differences among treatments were not significant.

Sensory panelists detected the differences in hardness and springiness among the treatments (Table 10). Generally, cheeses made from milk fat or randomized milk fat were rated harder than those made from HOSO except that the one made from HOSO with SCFA dissolved was rated as hard as the milk fat controls. A similar trend was shown from the instrumental texture profile analysis values for hardness (Table 9). Strugnell (8) indicated that the Cheddar cheese made with sunflower oil was soft and more crumbly than control cheeses made with milk fat. Whitehouse (14) found that modified HOSO cheeses were significantly harder, but more crumbly than the control cheese made with milk fat. Randomized milk fat cheese was slightly harder than the milk fat control cheese. The higher melting point of randomized milk fat might account for the increased firmness in cheese (38). Mohamed (11) observed that cheeses made from coconut oil were harder than those made from soy bean oil. Modified vegetable oil cheeses had sensory springiness scores similar to milk fat control cheese. Cheeses made from HOSO or HOSO with SCFA dissolved had the highest springiness scores, and were significantly higher than the cheese made from randomized milk fat. Strugnell (8) reported a rubbery texture of the cheese made from the rapeseed oil.

The correlations among sensory and TPA texture attributes are shown in Table 11. Among sensory texture attributes, only cohesiveness and springiness were correlated. Lakhani (26) and Raphaelides (25) also reported correlation between sensory cohesiveness and springiness. Among instrumental texture attributes, cohesiveness was correlated to

springiness and negatively correlated to adhesiveness. Lakhani (26) also found high negative correlation between TPA cohesiveness and adhesiveness (r = -0.99) but no relation between TPA cohesiveness and springiness. TPA hardness, gumminess, and chewiness were highly correlated to each other. The same correlations were reported by Lakhani (26) and Raphaelides (25). Of the six instrumental texture attributes, only adhesiveness and cohesiveness were significantly correlated with the sensory texture attributes. The reason for lower correlation between sensory and instrumental results might caused by the difference in the sample itself. The cheeses used for TPA analysis were freshly ripened cheeses, and the cheeses used for sensory analysis were frozen and thawed because it was not possible to produce all cheeses at one time. Jack et al. (39) reported no correlation for cheddar cheeses between Instron and sensory parameters. Green et al. (40) found that instrumental measurement were not good predictors of sensory attributes. However, Lee et al. (41) and Chen et al. (42) reported significant correlation between sensory attributes and instrumental parameters for cheese. Their results can be attributed to the use of a wide variety of cheese types with big textural differences among samples. In this study, the texture differences among sample were relatively small.

Commercial feasibility of production of Swiss cheese from modified HOSO. The cost for synthesis SCTG from commercial SCFA is around $5.01/kg. This estimate is based on the current costs of C4, C8, and C10 free fatty acids (43), C6 fatty acid (KIC Chemicals, Inc., Armonk, NY), glycerol (43), p-toluenesulfonic acid (Sigma Chemical Co., St. Louis, MO), and toluene (Sigma Chemical Co., St. Louis, MO). The current price of HOSO (AC Humko, Memphis, TN) is $1.34/kg. Therefore, the cost of HOSO

interesterified with SCFA at 100% of the level in milk fat is calculated around $1.91/kg. If p-toluenesulfonic acid and toluene can be recycled during synthesis of SCTG and assuming there is 1% loss for each during recycling, the cost of SCTG could be reduced to $2.68/kg. Consequently, the cost for modified HOSO could be reduced to $1.67/kg. This estimate does not take into account processing cost that may add $0.10 to $0.20/kg to the cost of modified HOSO. The price of butterfat shifts continuously. During 1975 to 1996, the butterfat price reached a peak between 1981 to 1984 at $4.07/kg (44), which was more than double of the current cost of modified HOSO estimated above. In 1998, again the butterfat price went to extremely high and averaged $5.81/kg from July to October 1998 (45). So using vegetable oil instead of butterfat in cheese manufacture will have great economic benefit, especially in a year, such as 1998, that the butterfat price is extremely high. The cost of SCFA (based on the amount used in this study) for the HOSO with SCFA dissolved is less than 1 cent, and the cost of HOSO with SCFA dissolved will be almost the same as the cost for the HOSO. If successful, the cheese made from HOSO with SCFA dissolved would have even greater economic benefit compared to the cheese made from HOSO with SCFA interesterified since no other extra processing cost will be involved in order to produce this type of cheese except for the homogenization, and the money saved on raw material costs depends on the price difference between butterfat and HOSO. The prices of SCFA are relatively stable. From 1986 to 1998, the C4 price slowly increased from $0.445 to $0.57/lb, C8 price increased from $0.74 to $1.29/lb, and C10 price slowly increased from $0.62 to $0.82/lb (43). Since C6 fatty acid often come from the same vegetable oil source as C8, we may expect a similar price increasing rate for C6

as that of C8 fatty acid. In the future, with the commercialization of high-oleic soybean oil, the price of high-oleic vegetable oils would be expected to decrease and the cost for HOSO or modified HOSO could be further reduced. Unfortunately, modification of HOSO with SCFA simply dissolved did not improve the cheese flavor in this study. Further studies could be done by adding a larger amount of free SCFA or adding the SCFA at different stages to see if the flavor of vegetable oil cheese could be improved.

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Table 1. Procedure for manufacture of "Iowa-style" Swiss cheese (22)

Operation	Time (min)	Temperature (°C)	Remarks
Fill vat		32	Approx. 107 kg skim milk with 2.8% various fats
Add starter		32	For 100 kg milk, add 2 g lactic culture, 4.1 g L. bulgaricus, 240 g S. thermophilus, 14.6 g P. shermanii.
Ripening	30 min	32	
Rennetting	30 min	32	For 100 kg milk, use 19.8 ml rennet diluted at 594 ml with distilled water
Cutting	10	32	0.64 cm knives
Foreworking	10	32	
Whey Removal		32	26 kg whey removed
Cooking		41	Replace whey with 26 kg 145°F water
Stir-out	2 hr from start of cut	41	
Vat Press	30 min under whey, 30 min without whey		23 kg weight
Hoop press	Approx. 18 hr.	22-26 room	9 kg Wilson hoops
Brine salting	2 days	4 brine	
Drying	Few hours	4	
Packaging			Vacuum packaging
Cold room	10 days	4	
Warm room	Approx. 3 weeks	22 room	
Finished cooler	3 months from start day	4	

Table 2. The flavor characteristics of Swiss cheeses produced with various fats[a]

Cheeses	Sweet	Salty	Sour	Bitter	Volatile	Swiss flavor	Caramelized
HOSO Control	5.50[dc]	4.67[ab]	5.84[abc]	4.26	5.71[dc]	5.08[c]	3.33
MF Control	6.00[bc]	3.83[bc]	5.20[bc]	3.70	6.69[bc]	8.38[ab]	3.70
RDM MF Control	6.38[abc]	5.17[a]	6.99[a]	4.71	7.38[ab]	7.72[b]	3.19
Modified HOSO with 100% Commercial SCFA	7.55[a]	4.71[ab]	6.66[ab]	3.45	7.49[ab]	9.11[a]	3.77
Modified HOSO with 100% MF SCFA	7.21[ab]	4.92[a]	5.93[abc]	3.56	7.58[ab]	8.78[ab]	4.00
Modified HOSO with 120% Commercial SCFA	5.89[c]	4.93[a]	7.15[a]	4.38	8.73[a]	7.96[ab]	4.22
HOSO with SCFA Dissolved	4.57[d]	3.61[c]	4.57[c]	3.39	4.71[d]	5.17[c]	2.71

[a]Means of two replications and 14 panelists. Means within columns followed by the same superscript are not significantly different ($p \leq 0.05$).
HOSO = high-oleic sunflower oil; MF = milk fat; RDM MF = randomized milk fat; SCFA = short-chain fatty acids.

Table 3. Free fatty acids contents (mg/g) of Swiss cheeses made with various fats[a]

Cheeses	C2:0	C3:0	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	Pooled C18	Pooled C20
HOSO Control	2.269	6.921^{abc}	0.005^{e}	0.000^{c}	0.000^{c}	0.004^{b}	0.000^{c}	0.000^{c}	0.069^{d}	0.412^{d}	0.006^{d}
MF Control	3.206	8.974^{a}	0.123^{d}	0.008^{bc}	0.005^{c}	0.080^{b}	0.032^{b}	0.099^{b}	0.268^{b}	0.768^{cd}	0.043^{cd}
RDM MF Control	2.219	5.721^{bcd}	0.159^{cd}	0.064^{b}	0.078^{ab}	0.122^{b}	0.138^{a}	0.378^{a}	0.886^{a}	1.332^{bc}	0.055^{bcd}
Modified HOSO with 100% Commercial SCFA	2.079	3.682^{d}	0.268^{b}	0.132^{a}	0.076^{ab}	0.193^{b}	0.012^{bc}	0.017^{c}	0.190^{c}	3.041^{a}	0.129^{abc}
Modified HOSO with 100% MF SCFA	2.431	8.081^{ab}	0.203^{bc}	0.050^{bc}	0.036^{bc}	0.126^{b}	0.000^{c}	0.000^{c}	0.185^{c}	2.001^{b}	0.139^{ab}
Modified HOSO with 120% SCFA	3.203	7.740^{ab}	0.348^{a}	0.177^{a}	0.127^{a}	0.765^{a}	0.000^{c}	0.000^{c}	0.130^{cd}	1.170^{c}	0.172^{a}
HOSO with SCFA Dissolved	2.141	4.028^{cd}	0.019^{c}	0.001^{bc}	0.003^{c}	0.063^{b}	0.004^{c}	0.006^{c}	0.067^{d}	0.413^{d}	0.003^{d}

[a]Means of two replications. Means within columns followed by the same superscript are not significantly different ($p \leq 0.05$). HOSO = high-oleic sunflower oil; MF = milk fat; RDM MF = randomized milk fat; SCFA = short-chain fatty acids.

Table 4. Chemical analyses of Swiss cheeses made with various fats[a]

Cheeses	Moisture (%)	Fat (%)	Salt (%)	pH	TA (% lactic acid)	Non-fat solids (%)[b]
HOSO Control	40.76[a]	27.92[b]	1.99[a]	5.33[ab]	0.89[a]	31.32[b]
MF Control	39.44[bc]	28.42[ab]	1.59[c]	5.39[a]	0.78[bc]	32.15[b]
RDM MF Control	40.13[ab]	28.43[ab]	1.77[b]	5.30[ab]	0.81[abc]	31.44[b]
Modified HOSO with 100% commercial SCFA	40.64[ab]	26.20[bc]	1.78[b]	5.28[ab]	0.88[ab]	33.16[b]
Modified HOSO with 100% MF SCFA	38.27[c]	24.97[c]	1.80[b]	5.37[a]	0.78[bc]	36.76[a]
Modified HOSO with 120% commercial SCFA	39.47[bc]	27.86[b]	1.59[c]	5.16[c]	0.77[c]	32.67[b]
HOSO with SCFA dissolved	40.97[a]	30.73[a]	1.82[b]	5.23[bc]	0.81[abc]	28.30[c]

[a]Means of two replications. Means within columns followed by the same superscript are not significantly different ($p \leq 0.05$).
[b]Calculated values.
HOSO = high-oleic sunflower oil; MF = milk fat; RDM MF = randomized milk fat; SCFA = short-chain fatty acids; TA = titratable acidity.

Table 5. Principal component analysis for free fatty acids in cheeses made from various fats[a]

Fatty Acids	Factor 1	Factor 2	Factor 3
C 2:0	0.406	-0.276	0.805*
C 3:0	0.180	-0.251	0.852*
C 4:0	0.972*	0.070	-0.048
C 6:0	0.953*	0.072	-0.137
C 8:0	0.910*	0.246	0.033
C10:0	0.837*	-0.080	0.035
C12:0	-0.074	0.955*	0.259
C 14:0	-0.076	0.930*	0.310
C 16:0	0.042	0.967*	0.226
Pooled C 18	0.476	0.197	-0.593
Pooled C 20	0.768	-0.074	-0.095
C 22:0	-0.097	0.759	-0.264
Eigenvalue	4.42	3.55	2.04
Cumulative Variance (%)	36.83	66.39	83.41

[a]Values in the table were the factor loading indicating the contribution of each fatty acid to the factors.
*Fatty acids that were regarded as high loadings for each factor.

Table 6. Correlation coefficients among sensory flavor parameters for cheeses made from various fats[a]

Parameters	Sweetness	Saltiness	Sourness	Bitterness	Volatiles	Swiss flavor
Saltiness	0.312					
Sourness	0.380	0.716*				
Bitterness	-0.060	0.151	0.295			
Volatiles	0.652*	0.568*	0.648*	0.236		
Swiss flavor	0.805*	0.332	0.319	-0.188	0.737*	
Caramelized flavor	0.622*	0.172	0.227	0.064	0.724*	0.703*

[a]Correlation coefficients ≥ 0.532 were significant at 5% significant level (df=12); correlation coefficients ≥ 0.458 were significant at 10% significant level.
*Correlation coefficients that were significant at $p \leq 0.05$.

Table 7. Correlation coefficients among chemical parameters for cheeses made from various fats[a]

Parameters	Moisture (%)	Fat (%)	Salt (%)	pH	TA (% lactic acid)	Non-fat solids (%)	Factor1	Factor 2
Fat (%)	0.303							
Salt (%)	0.535*	-0.172						
pH	-0.075	-0.350	0.258					
TA (% lactic acid)	0.574*	-0.184	0.686*	-0.033				
Non-fat solids (%)	-0.700*	-0.893*	-0.124	0.297	-0.134			
Factor 1	-0.266	-0.500**	0.469**	-0.413	-0.197	0.501**		
Factor 2	0.071	0.072	0.277	-0.006	-0.047	-0.088	0.000	
Factor 3	-0.240	0.068	0.137	0.239	-0.124	0.063	0.000	0.000

Correlation coefficients ≥ 0.532 were significant at 5% significant level (df=12); correlation coefficients ≥ 0.458 were significant at 10 % significant level.

*Correlation coefficients that were significant at $p \leq 0.05$.

**Correlation coefficients that were significant at $p \leq 0.10$.

Table 8. Correlation coefficients between sensory flavor parameters and chemical parameters for cheeses made from various fats[a]

Parameters	Sweetness	Saltiness	Sourness	Bitterness	Volatiles	Swiss Flavor	Caramelized Flavor
Moisture (%)	-0.369	0.130	0.144	-0.326	-0.343	-0.380	-0.515**
Fat (%)	-0.762*	-0.569*	-0.549*	0.166	-0.595*	-0.645*	-0.507**
Salt (%)	-0.144	0.318	0.143	-0.134	-0.394	-0.482**	-0.455
pH	0.172	0.007	-0.033	-0.228	-0.192	0.030	-0.250
TA (% lactic acid)	0.118	0.417	0.217	-0.187	-0.005	-0.089	-0.113
Non-fat solids (%)	0.746*	0.365	0.343	0.030	0.608*	0.663*	0.623*
Factor 1	0.396	0.469**	0.526**	0.123	0.756*	0.639*	0.613*
Factor 2	0.253	0.276	0.343	0.411	0.190	0.247	-0.147
Factor 3	-0.346	0.137	0.042	0.462**	0.117	-0.135	-0.104

[a]Correlation coefficients ≥ 0.532 were significant at 5% significant level (df=12); correlation coefficients ≥ 0.458 were significant at 10% significant level.

*Correlation coefficients that were significant at $p \leq 0.05$.

**Correlation coefficients that were significant at $p \leq 0.10$.

Table 9. The texture characteristics determined by instrumental texture profile analysis for Swiss cheese made from various fats[a]

Cheeses	Hardness (N)	Adhesiveness (Ns)	Springiness	Cohesiveness	Gumminess (N)	Chewiness (N)
HOSO Control	15.81	0.70	0.92	0.88 [a]	13.87	12.77
MF Control	16.14	1.30	0.88	0.85 [bc]	13.63	11.90
RDM MF Control	20.16	1.95	0.87	0.84 [c]	16.80	14.61
Modified HOSO with 100% commercial SCFA	13.27	1.05	0.91	0.87 [ab]	11.52	10.50
Modified HOSO with 100% MF SCFA	12.73	1.17	0.89	0.87 [ab]	10.99	9.73
Modified HOSO with 120% commercial SCFA	14.81	0.65	0.89	0.86 [ab]	12.73	11.31
HOSO with SCFA dissolved	17.01	0.93	0.90	0.87 [ab]	14.78	13.25

[a]Means of two replications. Means within columns followed by the same superscript are not significantly different ($p \leq 0.05$). HOSO = high-oleic sunflower oil; MF = milk fat; RDM MF = randomized milk fat; SCFA = short-chain fatty acids.

Table 10. The sensory evaluation on texture characteristics of Swiss cheeses made from various fats[a]

Cheeses	Hardness	Cohesiveness	Springiness
HOSO Control	6.35[dc]	8.23	9.47[ab]
MF Control	8.24[ab]	8.32	8.78[ab]
RDM MF Control	8.87[a]	6.26	6.94[c]
Modified HOSO with 100% commercial SCFA	7.43[abc]	7.48	8.38[bc]
Modified HOSO with 100% MF SCFA	6.91[bcd]	7.71	8.61[ab]
Modified HOSO with 120% commercial SCFA	5.84[d]	7.97	8.09[bc]
HOSO with SCFA dissolved	8.79[a]	8.37	10.04[a]

[a]Means of two replications and 14 panelists. Means within columns followed by the same superscript are not significantly different ($p \leq 0.05$).
HOSO = high-oleic sunflower oil; MF = milk fat; RDM MF = randomized milk fat; SCFA = short-chain fatty acids.

Table 11. Correlation coefficients among texture parameters of Swiss cheeses made from various fats[a]

Parameters	S Hard	S Cohesive	S Springiness	TPA Hard	TPA Adhesive	TPA Springiness	TPA Cohesive	TPA Gumminess
S Cohesive	-0.332							
S Springiness	-0.039	0.690*						
TPA hard	0.401	-0.440	-0.252					
TPA Adhesive	0.592*	-0.708*	-0.567*	0.388				
TPA Springiness	-0.252	0.217	0.372	-0.290	-0.418			
TPA Cohesive	-0.553*	0.424	0.533*	-0.151	-0.624*	0.710*		
TPA Gumminess	0.355	-0.391	-0.186	0.994*	0.320	-0.221	-0.048	
TPA Chewiness	0.330	-0.362	-0.132	0.976*	0.267	-0.089	0.049	0.991*

[a]Correlation coefficients ≥ 0.532 were significant at 5% significant level (df=12); correlation coefficients ≥ 0.458 were significant at 10% significant level.

*Correlation coefficients that were significant at $p \leq 0.05$.

S = sensory; TPA = texture profile analysis.

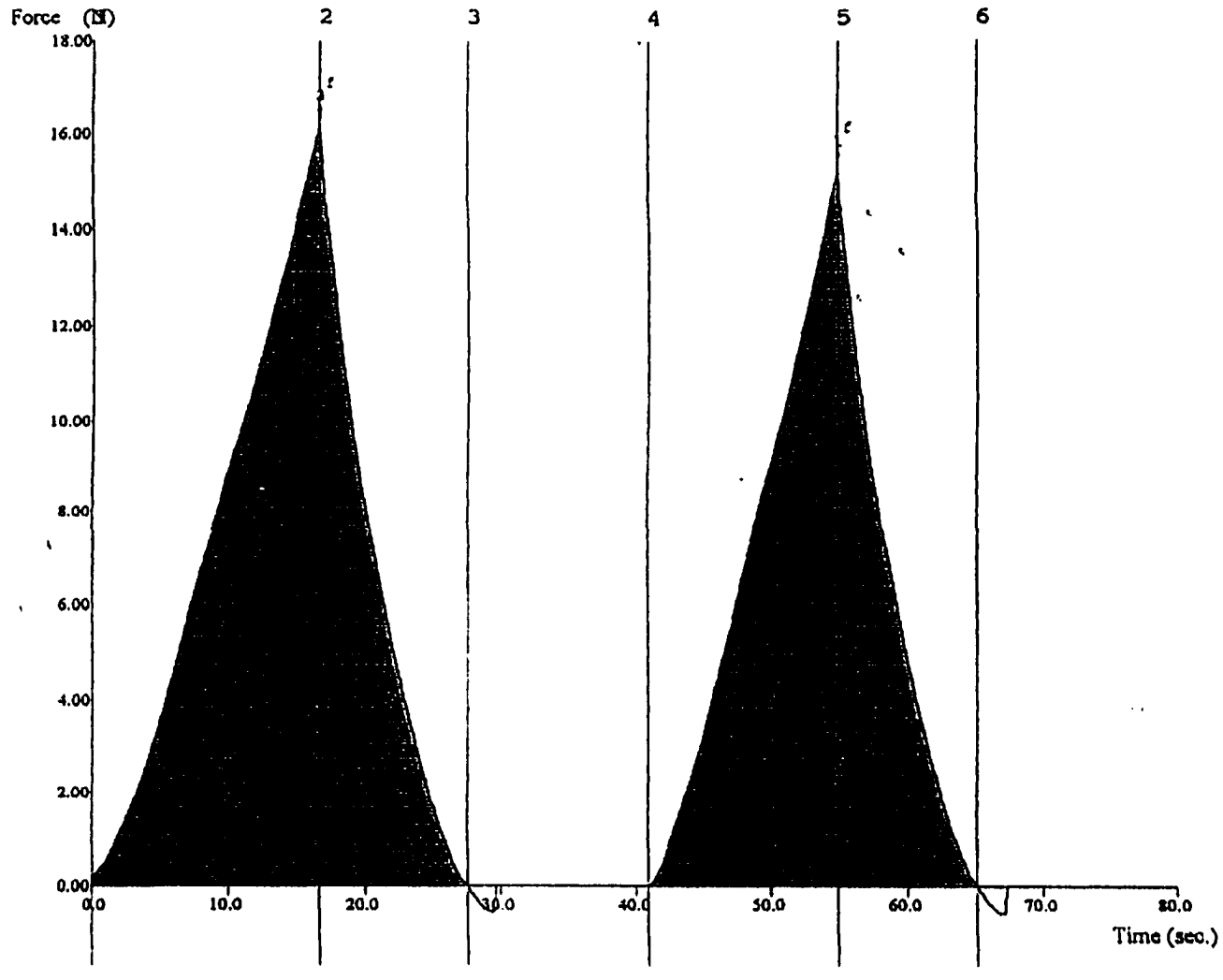

Figure. 1 Texture profile curve of Swiss cheese

CHAPTER 4. GENERAL CONCLUSIONS

In this study, the method of synthesis SCTG from SCFA and glycerol was improved by using toluene instead of benzene distillation to remove water from the reaction system. Toluene azeotrope distillation instead of benzene distillation to remove water from the reaction system during the synthesis of SCTG resulted in a faster and more complete esterification reaction. SCTG synthesized from commercial SCFA had a fatty acid composition that more closely resembled that of the milk fat; however, the SCTG from milk fat source gave better color and no bleaching was needed for this type of SCTG.

Better interesterification of SCTG with HOSO was achieved by increasing the sodium methoxide catalyst amount from 0.2% to 0.5% of total oil weight. The flavor problems encountered in the previous studies were probably caused by incomplete interesterification. The deodorization of modified vegetable oil at 180°C using a continuous pilot-scale deodorizer was sufficient, and no significant oil loss occurred, although some short-chain fatty acids might be lost during this process. After deodorization, all modified HOSO had very acceptable flavors with SCFA compositions closely resembling that of milk fat.

The GC method developed in this study for fatty acid analysis was satisfactory and simpler compared to the previous methods, and the method was suitable for free fatty acid analysis in cheeses as well.

Sensory flavor analysis results indicated that modification of HOSO with SCFA (C4-C10) significantly improved the cheese flavors compared with unmodified HOSO.

Swiss cheeses made from modified vegetable oils had flavors as acceptable as those from milk fat controls. No significant differences in any flavor attributes were found among three types of modified HOSO cheeses. Therefore, we can conclude that the source of SCFA and the exact amount of SCFA in the modified vegetable oils were not critical to achieving good flavors.

There were no significant differences in flavors between cheeses made from natural and randomized milk fat. The more randomized distribution of SCFA on triglyceride molecule in randomized milk fat did not cause a significant change in cheese flavor. However, randomized milk cheese was slightly harder than normal milk fat cheese. The harder texture of randomized milk fat cheese might caused by the higher melting point of randomized milk fat than normal milk fat.

All cheeses had a fat content within the normal range of Swiss cheese. This indicated that there was no significant fat loss during the cheesemaking procedures. The modified homogenization conditions of this study and the use of gum acacia as an emulsifier resulted in very stable fat in water emulsions.

Sensory and instrumental texture analysis indicated that the texture differences among the treatments were relatively small. No major texture defects were found in vegetable oil cheeses although vegetable oil cheeses were generally softer than milk fat cheeses. The lower correlation between sensory and instrumental results might be attributable to the use of different samples in two methods; that is, fresh cheeses were used for texture profile analysis and frozen and thawed cheeses were used in the sensory

analysis. If so, the freeze-thaw cycling might affect the texture of milk fat cheese more than that of vegetable oil cheese.

Dissolving SCFA (C4-C10) in HOSO did not improve the cheese flavors and the SCFA (C4-C10) were not present in the final cheese product. The reason for this loss of SCFA was not clear, but these results suggested that added SCFA were metabolized during the cheese ripening and must be continuously replaced by hydrolysis of the cheese triglyceride.

The raw material cost for modified HOSO interesterified with 100% commercial SCFA was estimated around $1.91/kg. If p-toluenesulfonic acid and toluene can be recycled during synthesis of SCTG, the cost for modified HOSO could be reduced to $1.67/kg. If modification of HOSO by dissolving SCFA in oil works, the cost will be even lower and no extra processing cost will be involved except for homogenization.

This work confirmed that nutritionally superior Swiss cheese with good flavor and texture could be produced from modified vegetable oils. Further work could be done by using other types of vegetable oils, such as soybean oil and high-oleic soybean oil. The technology developed in this study could be employed to the production of other types of cheese, in which SCFA are an important flavor factor. Eventually, low-fat or reduced-fat vegetable oil cheeses could be made using the same technology. Since modification of HOSO with SCFA dissolved in oil has great economic benefit, more studies can be done in this area, such as using a larger amounts of SCFA and adding the SCFA at different processing stages to see if the flavor of the vegetable oil cheese could be improved.

APPENDIX A. SENSORY FLAVOR SCORECARD

Respondent Code: _____ Date: _____

Place a mark perpendicular to the horizontal line corresponding to the intensity of the attributes. Label the mark with the sample code number.

SWEET

None Strong

SALTY

None Strong

SOUR

None Strong

BITTER

None Strong

VOLATILES

None Strong

TYPICAL SWISS CHEESE FLAVOR

None Strong

CARAMELIZED FLAVOR

None Strong

COMMENTS:

APPENDIX B. SENSORY TEXTURE SCORECARD

Respondent Code: _____ Date: _____

Place a mark perpendicular to the horizontal line corresponding to the intensity of the attributes. Label the mark with the sample code number.

HARDNESS – Place sample between molar, bite through it once, evaluate the force required to bite into the cheese

Soft Hard

COHESIVENESS – Place sample between molars, compress fully, note the degree to which sample deforms rather than crumbles or breaks

Breaks Deforms

SPRINGINESS – Place sample between molars, compress partially without breaking the sample structure, release and evaluate the degree to which the sample returns to its original shape

Not springy Very springy

COMMENTS:

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IMAGE EVALUATION
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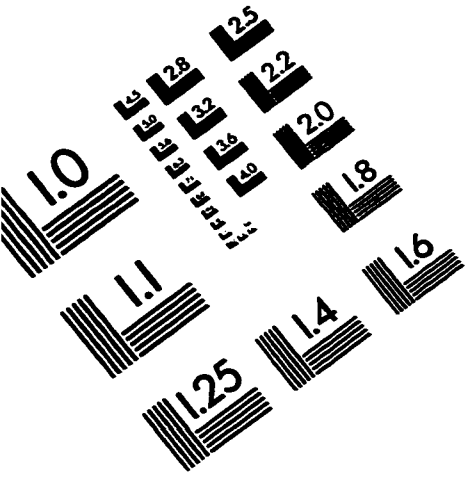

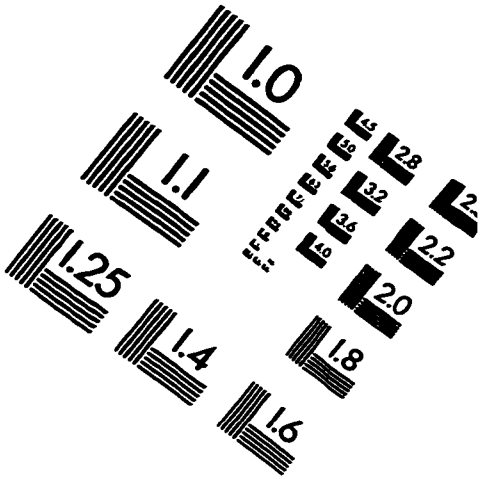

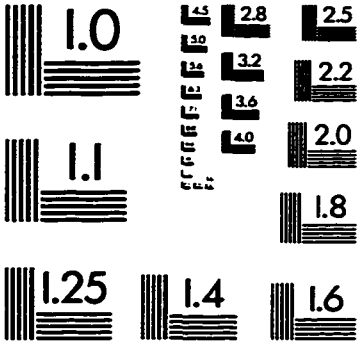

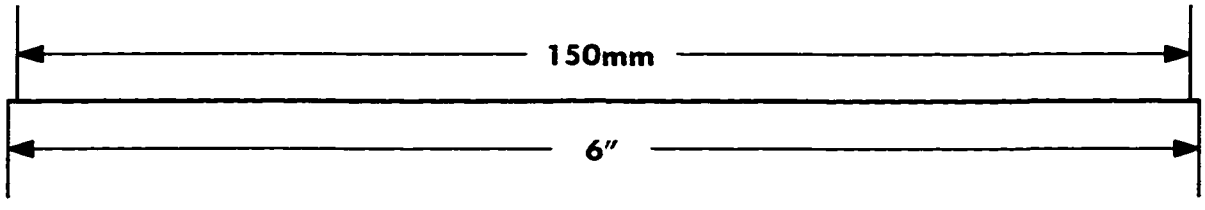

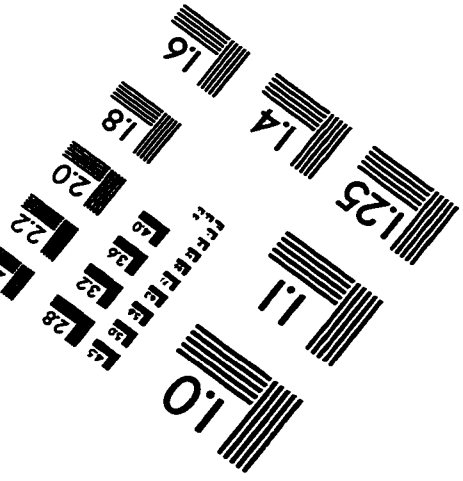

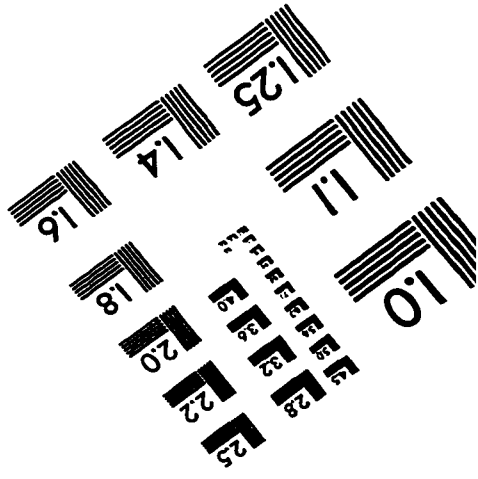

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