THE INFLUENCE OF FREE FATTY ACIDS ON AGE AND FLAVOR CHARACTERISTICS OF COMMERCIAL CHEDDAR CHEESE AND COMPOSITION ANALYSIS USING NEAR INFRARED SPECTROSCOPY

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LITERATURE REVIEW

Source of free fatty acids

The free fatty acids (FFA) in cheese are derived from two major sources: (1) breakdown of the fat by lipolysis and (2) metabolism of carbohydrates and amino acids by bacteria (Aston and Dulley, 1982). Nakae and Elliott (1965) demonstrated that the FFA from acetic to caproic were produced from casein hydrolysates, but the bulk of the evidence indicates that lipolysis is the principal contributor of free fatty acids of chain length C_4 and greater (Ohren and Tuckey, 1969; and Foda *et al.*, 1974). Lipolysis, which can be defined as the enzymatic hydrolysis of fat, has economic significance, in that it can result in the accumulation of either desirable or undesirable end products. Bills and Day (1964) reported that the percentages by weight of the individual FFA from caproic through linolenic in experimental Cheddar cheese were very close to the values reported by Herb *et al.* (1962) and Jack (1960) for the same esterified fatty acids of milk fat. This suggests that these acids may be hydrolyzed from milk fat in a nonselective manner by milk lipase, microbial lipase or both. Natural milk lipase, contaminant bacteria in the cheese milk, starter bacteria and contaminant bacteria in the cheese can all ultimately influence the amount of FFA liberated in the cheese.

Intrinsic milk lipase

The level of free fatty acids in dairy products may be due to pre-manufacture lipolysis arising from the uptake from milk of FFA liberated by native milk enzyme (Downey, 1975).

In 1957 Tarassuk and Frankel reported that milk contained at least two different lipase systems. The first system is membrane lipase, which is irreversibly absorbed on the fat-globule membrane material when freshly drawn milk is cooled. Tarassuk and Frankel (1957) reported that the membrane lipase is responsible for spontaneous lipolysis. The term spontaneous is used to describe milks which develop lipolysis without any apparent mechanical agitation. Spontaneous lipolysis is initiated by prompt cooling of fresh milk. Free fatty acids accumulate following relatively short holding periods regardless of whether the milk is subsequently kept cold or warm (Jensen, 1964). The animal factors which predispose milk from individual cows to develop lipolysis spontaneously without mechanical agitation are elevated level of blood-derived constituents in milk, late lactation, poor nutrition, low milk yield, estrous cycle, hormonal control and mastitis (Deeth and Fitz-Gerald, 1976).

The other lipase system is plasma lipase, which is associated with casein (Tarassuk and Frankel, 1957). The plasma lipase which is responsible for induced lipolysis requires homogenization, agitation or temperature manipulations for activation (Chandan and Shahani, 1964). Such activation treatments apparently do not affect the enzyme, but they do seem either (a) to facilitate the release of lipase

from casein micelle or (b) to promote the adsorption of the enzyme on the fat globules or (c) to alter the orientation of the adsorbed fat globule membrane (Jenness and Patton, 1959). Once induced, lipolysis proceeds rapidly but only for a relatively short period, following which it levels off and no further accumulation of FFA is observed despite the presence of excess triglyceride substrate. The decrease in activity over time of induced lipolysis in milk is attributed to the accumulation of inhibitory FFA at the fat globule interface and failure of the enzymes to desorb from the interface leading to a slowing-down and ultimate inhibition of lipolysis (Downey, 1980).

Inactivation of intrinsic milk lipase

Milk lipase is relatively unstable and can be inactivated by salt, acid, light, oxidation and heat. Normal pasteurization of market milk, which inactivates the enzyme, prevents further breakdown of the fat due to lipase but does not destroy any FFA which are already present at pasteurization (Deeth and Fitz-Gerald, 1976).

Bacterial lipolysis in cheese-milk

Many of the microorganisms which contaminate dairy products are lipolytic, *i.e.*, they produce lipase. The most common source of the lipase are psychrotrophic bacteria which grow at refrigeration temperatures. The number of these organisms in milk increases during storage and may produce significant amounts of lipase after about three days. Many bacterial lipases differ from milk lipase, because they are not inactivated by pasteurization even though the organisms that produce them are

destroyed. They can therefore be carried through in an active form into manufactured products (butter, cheese) and cause fat breakdown during storage of these products (Deeth and Fitz-Gerald, 1976).

Pinheiro *et al.* (1965) have shown that extracellular lipase from psychrotrophic raw milk bacteria (*Pseudomonas* and *Alcaligenes* sp.) are resistant to pasteurization temperatures which kill the bacteria themselves. The lipase from *Pseudomonas fluorescens* and *P. fragi* remained wholly or partly active after heat treatment at 63 °C for 30 min (Law *et al.*, 1976). Ohren and Tuckey (1969) observed that high levels of FFA developed during maturation in cheese made from heat-treated milk if levels of organisms were high in the raw milk, indicating that surviving bacterial lipase were also active in cheese.

Lipolysis in cheese by starter bacteria

During cheese-making a slight increase of butyric and longer fatty acids occurred in every cheese made with starter and further small increases occurred during ripening. However, there was no increase in the content of these acids in the cheese made with δ-gluconolactone which was used as an acid-producing agent, indicating that lactic acid bacteria were weakly hydrolyzing the milk fat (Reiter *et al.*, 1967). These results are consistent with the finding of Fryer *et al.* (1967) that lactic acid bacteria are capable of weakly hydrolyzing cheese fat. It was apparent that not only the single-strain but also the component bacteria of multiple-strain starters were capable of such hydrolysis. Some starter streptococci appeared to be

more lipolytic than others but none was as active as the leuconostoc and *S. diacetilactis*. This was consistent with earlier results, which indicated that commercial starters were more lipolytic than single-strains (Reiter *et al.*, 1967).

Franklin and Sharpe (1963) in their lots of experimental cheese found higher flavor scores in those lots with the greater number of lipolytic bacteria, and visa versa.

Lipolysis in cheese by contaminant bacteria

In dairy products microbial growth is frequently associated with post manufacture lipolysis (Downey, 1980). Fryer *et al.* (1967) indicated that an important part of the cheese microflora is derived from the air or from dairy equipment during cheese-making. Free fatty acid concentrations were higher in reference flora cheeses than in controls. The reference flora cheese contained non-starter bacteria isolated from cheese curd at commercial creameries, while the control cheese was made in an aseptic vat with starter (Law *et al.*, 1976). As observed in an earlier study only the non-starter lactic acid bacteria increased in numbers during the latter stages of maturation (Fryer *et al.*, 1967).

FFA extraction

Milk fat FFA (C_4 - $C_{18:1}$) vary widely in solubility and therefore their quantitative removal from the fat by simple solvent partitioning is very difficult (Deeth *et al.*, 1983). Several methods have been designed which utilize a liquid-solid partitioning. The two most common solid supports which have been used for

this purpose are silicic acid rendered alkaline with KOH (Harper *et al.*, 1956; McCarthy and Duthie, 1962; Iyer *et al.*, 1967; Woo and Lindsay, 1980, 1982) and anion exchange resins (Hornstein *et al.*, 1960; Bills *et al.*, 1963).

The alkaline arrestant columns have been used for isolation of FFA from cheese, but the original columns induced extensive glyceride hydrolysis which invalidates the data (Woo and Lindsay, 1980). Woo and Lindsay (1982) adapted a modified version of the alkaline arrestant column for quantitative FFA isolation from unaltered cheese for gas chromatographic quantification. This method reduced the possibility of glyceride hydrolysis, but still required some preparation or pretreatment of the cheese. The method also requires the removal of lactic acid because of its co-elution with the C_{10} and C_{12} during gas chromatography.

The anion exchange resins used for isolation of FFA from fat also have been criticized, because they give incomplete recovery, induce glyceride hydrolysis and are time-consuming. Methods employing fat isolates yield FFA profiles that are different from those in intact sample because of partitioning properties of FFA.

Deeth *et al.* (1983) devised an extraction procedure for gas chromatographic determination of FFA in dairy products. In their procedure the lipid portion of the cheese is removed through extraction with diethyl ether. Hexane is added to the mixture and the total volume of the solution is added to a small glass chromatography column containing de-activated neutral alumina. Alumina is used in this method because of its high affinity for acids and the simplicity of its use.

Deeth *et al.* (1983) found that considerable glyceride hydrolysis occurred on highly active alumina but that negligible hydrolysis occurred when the alumina was deactivated with 4 - 6 percent water. The deactivated alumina efficiently removes the FFA. The triglycerides are washed from the alumina column with hexane - diethyl ether (1:1). The alumina with absorbed FFA is vacuum dried and the FFA are selectively removed from the alumina with a diisopropyl ether - 6 % formic acid mixture. The formic acid efficiently releases the FFA from the alumina. The procedure is relatively simple and convenient for measuring individual FFA in a wide range of dairy products. In contrast to other methods the procedure requires only small quantities of sample, solvent and other materials per assay. Hydrolysis of milk fat glycerides is negligible and quantification of all major FFA with little interference from lactose is possible.

FFA separation and quantification

After extraction of the FFA from the cheese, they can be separated and quantified by gas chromatography of their methyl esters (Bills *et al.*, 1963), butyl esters (Iyer *et al.*, 1967) or the underivatized FFA (Woo and Lindsay, 1980, 1982; Deeth *et al.*, 1983). Deeth *et al.* (1983) obtained excellent resolution of the underivatized FFA using the stationary phase SP-216-PS (Supelco, Inc., Bellefonte, PA). Woo and Lindsay (1982) found an ethylene glycol succinate (DEGS-PS) stationary phase (Supelco, Inc., Bellefonte, PA) which has several desirable features for separation of underivatized FFA analysis. Since the free fatty acids can now be

successfully separated on several stationary phases, a derivatization step was considered unnecessary. Deeth *et al.*, (1983) considered the derivatization step as another source of possible error.

Rapid FFA assessment

Ikins *et al.* (1988) compared three relatively rapid methods of determining total FFA concentrations in cheese to the results of the gas chromatographic (GC) method described by Deeth *et al.* (1983). Acid Degree Value (Richardson, 1985) results correlated poorly with the GC data, particularly for the short-chain fatty acids. Values obtained using the Copper Soap Method (Bynum *et al.*, 1984) correlated closely with the GC data for total and long chain fatty acids, but did not correlate as well with short-chain fatty acid levels. The Extraction-Titration Method (Salih *et al.*, 1977) was found to be a simple and reliable technique and yielded values which correlated closely with short-chain fatty acid concentrations.

FFA content of Cheddar cheese

Many investigators utilizing various methodologies have reported various concentrations of FFA in Cheddar cheese. Generally, young Cheddar cheese contains low levels of FFA and good quality aged Cheddar cheese has intermediate concentrations of individual FFA (Woo and Lindsay, 1984). Some work in the area of Cheddar cheese flavor has been conducted on cheeses of all ages and other work has been on cheeses of a particular age. Table 1 represents a sample of the various concentrations of FFA reported by selected authors.

For the data in Table 1, Bills and Day (1964) used an anion exchange method to separate the free fatty acids in Cheddar cheese. The results of Woo and Lindsay (1982) were obtained using a silicic acid column rendered alkaline. Lin and Jeon (1987) and Marsili used the procedure described by Deeth *et al.* (1983). In most cases when cheeses of different ages were studied the concentration of the individual FFA increased with increasing age of the cheese sample.

Cheddar cheese flavor

Unripened cheese is known as "fresh", "young" or "green" cheese. This cheese has a flat, curdy flavor and a tough, corky body. Cheese that has been properly cured for about three months has a mild, slightly nutty Cheddar cheese flavor and is therefore referred to a "mild" cheese. At six to eight months of age more of the Cheddar flavor should be present. Such cheese may be considered a "semi-aged" or "medium" cheese. Generally, about a year is required to develop the full Cheddar cheese flavor desired in "aged" or "sharp" cheese. "Extra-sharp" cheese needs to cure a year or more. This grade of cheese gives strong Cheddar flavor without lipolyzed flavor (Nelson and Trout, 1981).

Influence of fat on Cheddar cheese flavor

Of the various milk constituents, lipids have the greatest effect on the flavor of dairy products. They serve as solvent and precursor of both desirable and undesirable flavors. Chemical and biochemical reactions occurring during the

Table 1. The FFA content of Cheddar cheeses of various ages as reported by several investigators.

Investigators	Months	FFA (ppm)								
		C_4	C_6	C_8	C_{10}	C_{12}	C_{14}	C_{16}	$C_{18:0}$	$C_{18:1}$
A	3-4	76	29	36	55	87	191	516	104	319
	7-12	127	33	43	53	83	236	510	192	458
B	4	69	31	37	69	88	211	542	232	1027
C	3	20	12	8	30	46	139	371	116	292
D	3	13	8	<5	16	31	112	274	140	360
	6	8	8	<5	23	46	152	373	161	404
	10	23	18	19	50	48	226	452	160	416
	12	46	50	11	64	63	265	500	179	486
E	6	15	2	6	25	37	103	285	524*	--
	12	111	33	38	67	68	183	397	131*	--

* C_{18} Congeners

A. Bills and Day (1964)
B. Deeth *et al.* (1983)
C. Lin and Jeon (1987)
D. Marsili (1985)
E. Woo *et al.* (1984)

manufacture, storage and utilization of dairy products convert lipid components into a multitude of organic compounds (Day, 1966).

Ohren and Tuckey (1965) found improved cheese flavor as the fat content of the milk increased. Many authors have documented that skim milk cheese has considerably lower levels of free fatty acids than normal cheese (Patton, 1963; Ohren and Tuckey, 1965; Deane, 1972; Deane and Dolan, 1973; Foda *et al.*, 1974; Dulley and Grieve, 1974), implicating lipolysis as the major source of FFA in normal Cheddar cheese. Ohren and Tuckey (1969) stated that only Cheddar cheese containing 50% fat or more in the dry matter developed a typical flavor, whereas cheese with less than 50% fat did not. Cheddar cheese manufactured with vegetable or mineral oils also appear not to develop characteristic cheese flavor. The fat phase may act as a reservoir for fat soluble precursors and intermediates (Foda *et al.*, 1974). Typical Cheddar flavor does not develop when fat hydrolysis is either low (Kristofferson and Gould, 1960) or excessive (Hlynka *et al.*, 1943).

Influence of FFA on Cheddar flavor

Patton (1963) evaluated the contribution of various classes of compounds to Cheddar cheese aroma by adding reagents to selectively block functional groups and concluded that acetic, butyric, caproic and caprylic acids constitute the backbone of Cheddar cheese aroma. Short-chain fatty acids, which may arise from lipolysis of milk fat, play a significant role in the flavor of Cheddar cheese (Kristofferson and Gould, 1958; Ohren and Tuckey, 1965; Ohren and Tuckey, 1969).

Peterson and Johnson (1949) noted that FFA of intermediate chain length were produced during the ripening process and were characteristic of Cheddar cheese flavor. Arbige *et al.* (1986) suggested that the total concentration of C_4, C_6 and C_8 FFA is an important factor for flavor development during cheese ripening. Lin and Jeon (1987) suggested that the total concentration of C_4 and C_6 FFA may be a good indicator of flavor development during cheese ripening. The longer-chain FFA are believed to contribute an overall cheese background flavor (Bills and Day, 1964; Forss, 1969). Marsili (1985) reported that the best indicators of lipolytic age were the combination of the FFA C_{10}, C_{12}, C_{14} and C_{16}.

Influence on flavor by FFA and other compounds

Previous investigations have shown that lipolysis during aging of Cheddar cheese is normal and desirable but is not the sole reaction necessary for flavor production (Ohren and Tuckey, 1969). The flavor of Cheddar cheese was found to be related more to the free fatty acid concentration and hydrogen sulfide concentration ratio than to any other compounds of combinations of compounds (Kristofferson and Gould, 1960). Results of Ohren and Tuckey (1969) showed that not only an optimum level exists for the concentration of FFA plus acetate, but these compounds should be present within a certain range for desirable flavor development.

FFA and flavor defects in Cheddar cheese

Generally, young Cheddar cheese contains low levels of FFA while aged desirably-flavored Cheddar cheese has intermediate concentrations of individual FFA. However, if conditions permit development of excess FFA in Cheddar cheese, rancid off-flavors are readily apparent (Woo and Lindsay, 1984). In two rancid samples Bills and Day (1964) observed a tenfold increase in the concentrations of the individual acids from butyric through linolenic. Kristofferson *et al.* (1958) observed high concentrations of free fatty acids that were associated with cheese of less desirable flavor characteristics.

The concentrations of specific FFA had an important influence on the flavor of cheese, because in those cheeses which had abnormally high amounts of C_{10}, C_{12} and C_{14} acids the samples had unclean and rancid flavors. In the cheeses having the most desirable flavor the concentration of C_{10}, C_{12} and C_{14} acids were always much lower than in those cheeses having fermented, unclean and rancid flavors (Ohren and Tuckey, 1969). Large amounts of FFA especially the C_{10} and C_{12} FFA cause soapy flavor(Woo and Lindsay, 1982).

Near Infrared Reflectance Spectroscopy

The near infrared region is composed of radiation with wavelengths of 700-3000 nm, but wavelengths in the region of 1,000 to 2,600 nm are used in most applications (Hruschka,1987). Near-infrared spectroscopy is based on the actual number of molecules of an individual constituent (Murray and Williams, 1987) and

functions in two modes; namely reflectance and transmission. In the transmission mode a lead sulfide cell is positioned directly beneath the sample cell and an empty quartz transmission cell is used. In the reflectance mode, the light beam is directed down towards the sample. Light reflected off the sample is then picked up by four lead sulfide cells equally spaced at 45 degrees above the sample. A ceramic disk is used as a reference in the reflectance mode. The detected signal is fed into a log amplifier, digitized, and sent to a computer (Lanza, 1983). The data is recorded as log 1/R (R = reflectance) which varies approximately linearly with the concentration of the absorber (Norris *et al.*, 1976).

Rapid analysis of milk for fat, protein and total solids content by infrared reflectance (IR) absorption spectroscopy already is having a significant impact on the dairy industry (McGann, 1978). Infrared milk analysis is an approved AOAC method for determination of water, protein and lactose in milk (Biggs, 1972). In New Zealand, near infrared reflectance (NIR) instruments are currently authorized for official use on whole milk powder, buttermilk powder, skim milk powder, casein, caseinates and whey protein concentrates. These products can be analyzed for fat, moisture, protein, lactose, acid and ash using NIR (Woollard, 1985).

Landa (1979) and McClure and Hamid (1980) reported that computer assisted spectrophotometric equipment necessary for applying near infrared reflectance analysis to food materials was generally available. Reflectance spectroscopy using the near infrared spectrum was used to determine the oil,

protein and moisture content of grains and oilseeds (Ben Gara and Norris, 1968; Hymowitz *et al.*, 1974; Rinne *et al.*, 1975). Similar instruments were used to estimate the nutritional quality of forages (Norris *et al.*, 1976; Shenk *et al.*, 1979) and degree of ripeness of fruits (Chen, 1978).

Frank and Birth (1982) found high correlations between reflectance at specific wavelengths and protein, fat and moisture content in cheese, indicating a potential use for reflectance spectroscopy for measuring cheese composition. Their correlation coefficients for reflectance with constituent concentration in cheese were .942 for fat, .938 for protein and .965 for moisture. The reflectance at the selected wavelengths was linear to constituent concentrations.

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ABSTRACT OF THE RESEARCH

Thirty-nine commercial Cheddar cheeses of various ages and from several manufacturers were analyzed by a gas chromatographic technique for free fatty acids (FFA) content. The cheese samples were also evaluated by a trained taste panel for age characteristics, flavor defects and body and texture defects. Stepwise regression analysis was utilized to develop a regression equation which indicated the taste panel age score using the C_6, C_{16} and C_{18} FFA concentrations (ppm). The multiple correlation coefficient for the panel age regression equation was .805 ($p \leq .05$).

Stepwise discriminant analysis was utilized with the FFA concentrations to classify the samples into age categories. The C_6, C_{16} and C_{18} FFA concentrations were selected as the best parameters to reflect the age categories. Canonical 0discriminant analysis was used to develop a canonical function and a canonical score was calculated for each sample. Prediction ranges were to allow the estimation of a sample age. The samples age was predicted on the basis of the canonical score calculated by the canonical function using the concentration of the C_6, C_{16} and C_{18} FFA. Results of discriminant analysis performed on the canonical scores for the samples indicated that 83 percent of the mild, 83 percent of the medium samples, 55 percent of the sharp and 80 percent of the extra-sharp samples were classified correctly.

A regression equation was developed and related the panel acid score to the FFA concentrations of the samples. A combination of C_8 and C_{10} FFA

concentrations was selected as the best indicator of the taste panel acid score. The multiple correlation coefficient for the acid regression equation was .673 ($p \leq .05$). The concentrations of C_{12} and C_{14} could also be good indicators of Cheddar cheese acid because they were highly correlated to C_8.

Composition analysis of cheese was determined using the Babcock procedure for fat, vacuum oven procedure for moisture (AOAC), and the semi-micro-Kjeldahl procedure for protein (AOAC). The results were compared to composition analysis by near infrared reflectance spectroscopy (NIRS). The correlation coefficients (r) between the chemical and NIRS determination were .616, .817 and .835 for fat, moisture and protein, respectively. The correlation coefficients were not as high as expected, but could be improved with a larger and more detailed calibration set. The NIRS exhibited the capability of rapid composition analysis although the correlation coefficients require improvement before the expected precision can be obtained.

INTRODUCTION

Free fatty acids and Cheddar cheese flavor

The development of flavor and aroma in Cheddar cheese depends, to a great extent, on a complex balance of organic chemicals produced during ripening. The Dutch investigator Mulder (1952) suggested the "Component Balance Theory". The theory stated that characteristic cheese flavor is not related to any single compound but to a mixture of compounds resulting from the degradation of fat, protein and lactose. When these compounds occur in a certain specific balance, characteristic cheese flavor results.

Of the various milk constituents, lipids have the greatest effect on the flavor of cheese. They serve as solvent and precursor of both desirable and undesirable flavors. Chemical and biochemical reactions occurring during the manufacture and storage of cheese convert lipid components into a multitude of organic products (Day, 1966).

The free fatty acids (FFA) in cheese are derived from two major sources: (1) breakdown of the fat by lipolysis and (2) metabolism of carbohydrates and amino acids by bacteria (Aston and Dulley, 1982). The bulk of the evidence indicates that lipolysis is the principal contributor of FFA of chain length C_4 and greater (Ohren and Tuckey, 1969; and Foda *et al.*, 1974). Ohren and Tuckey (1969) found that cheese flavor improved as fat content of the cheese milk increased. Many authors have documented that skim milk cheese has considerably lower levels of FFA than

normal cheese and that skim milk cheese lacks the flavor intensity of normal cheese (Patton, 1963; Ohren and Tuckey, 1965; Deane, 1972; Deane and Dolan, 1973; Foda *et al.*, 1974; and Dulley and Grieve, 1974).

Patton (1963) evaluated the contribution of various classes of compounds to Cheddar cheese aroma by adding reagents to selectively block functional groups and concluded that acetic, butyric, caproic and caprylic acids constitute the back bone of Cheddar cheese aroma. Short-chain FFA, which may arise from lipolysis of milk fat, have been shown to play a significant role in the flavor of Cheddar cheese (Kristofferson and Gould, 1958; Ohren and Tuckey, 1965; Ohren and Tuckey, 1969).

Near infrared reflectance composition analysis

Landa (1979) and McClure and Hamid (1980) reported that computer assisted spectrophotometric equipment necessary for applying reflectance analysis to food material was generally available. Reflectance spectroscopy using the near infrared spectrum was used to determine the oil, protein and moisture content of grains and oilseeds (Ben Gara and Norris, 1968; Hymowitz *et al.*, 1974; Rinne *et al.*, 1975). Similar instruments were used to estimate the nutritional quality of forages (Norris *et al.*, 1976; Shenk *et al.*, 1979) and degree of ripeness of fruits (Chen, 1978).

Rapid analysis of milk for fat, protein and total solids content by infrared reflectance (IR) absorption spectroscopy already is having a significant impact on

the dairy industry (McGann, 1978). Infrared analysis is an approved AOAC method for the determination of moisture, protein and lactose in milk (Biggs, 1972). In New Zealand, near infrared reflectance spectroscopy (NIRS) instruments are currently authorized for official use on whole milk powder, buttermilk powder, skim milk powder, casein, caseinates and whey protein concentrates. These products can be analyzed for fat moisture, protein, lactose, acid and ash using NIRS (Woollard, 1985).

The objectives of the present work were to obtain individual FFA profiles as well as sensory characteristics for commercial Cheddar cheese samples and to determine if a statistical relationship exists between sensory characteristics and the concentration of FFA individually or in combination. In addition the composition analysis for the cheese samples were compared between the conventional chemical analyses and NIRS measurements for fat, protein and moisture.

MATERIALS AND METHODS

Samples

Thirty-nine commercial Cheddar cheese samples of various ages were collected from local supermarkets. Eleven mild, seven medium, fifteen sharp and six extra-sharp samples were tested. The cheeses were from several manufacturers who employed different manufacturing and ripening procedures. The samples were stored at -18 F until analysis. Immediately before analysis, a representative sample was grated for 30 sec in a food processor.

Composition Analysis

The samples were analyzed for moisture and protein utilizing methods described by the Association of Official Analytical Chemists (AOAC, 1985). The fat content of the cheese was determined utilizing the Babcock procedure as described by Richardson (1985). Moisture content was determined by vacuum oven at 100 C for 5 h with 68 cm of vacuum. The Buchi semi-micro-Kjeldahl procedure was utilized to determine the percent nitrogen and a factor of 6.38 was used to determine the percent protein of the samples. All composition tests were conducted in triplicate.

The samples were also scanned with a Pacific Scientific 4250 Near Infrared Scanner, equipped with three tilting filters which provided a continuous scan (291

data points) from 1900 to 2320 nm. The near infrared spectroscopy (NIRS) spectral data were stored as Log 1/R, where R is percent reflectance.

The laboratory values for each sample were compared by the instrument statistical software (Infrasoft International, Pennsylvania State University) with the NIRS spectral data for each of the samples. Multiple step-wise linear regression was used to select the wavelengths and calibration coefficients which provided the best statistics: highest correlation coefficient and lowest standard error of calibration (SEC) and highest correlation coefficient and standard error of prediction (SEP) (Dubois *et al.*, 1989).

Sensory analysis

Two training sessions were conducted on a weekly basis to familiarize the panel with the scoring procedures. The sensory panel comprised of eight members of the Kansas State University dairy products judging team evaluated the cheeses for aged flavor, flavor defects and body and texture defects. The score card used to evaluate the samples was adapted from the American Dairy Science Association standard score card (Figure 1). Aged flavor was ranked on a scale of 1 - 5: where 1,2,3,4 and 5 represented young, mild, medium, sharp and extra-sharp, respectively. The intensity of the flavor defects was ranked on a scale of 1 - 5; where 1,2,3,4 and 5 indicated none, slight, moderate, definite and extreme defect, respectively. The body and texture defects were evaluated using the same scale as used for flavor defects. In all evaluations the judgements were made to the nearest one point.

Figure 1. Score card used for the evaluation of commercial Cheddar cheese.

Name:_____ Date:_____

Sample ID Number									
Intensity of Flavor Development									
Flavor Defects 1 = None 2 = Slight 3 = Moderate 4 = Definite 5 = Extreme	Acid								
	Bitter								
	Fermented\Fruity								
	Flat\Lacks Flavor								
	Rancid								
	Sulfide								
	Unclean								
	Whey Taint								
	Other								
Body & Texture Defects 1 = None 2 = Slight 3 = Moderate 4 = Definite 5 = Extreme	Corky								
	Crumbly								
	Curdy								
	Gassy								
	Mealy								
	Open								
	Pasty								
	Short								
	Weak								

Intensity Scale: 1 = young
2 = mild
3 = medium
4 = sharp
5 = extra-sharp

Others could include: a - Feed
b - Garlic
c - Heated
d - Moldy
e - Yeasty

FFA Extraction

The method used to extract the FFA from the cheese samples was similar to the procedure described by Deeth et al. (1983). A representative aliquot of Cheddar cheese was grated in a Black and Decker Food processor. One gram of sample was transferred into a 16 x 150 mm test tube. Five mL of redistilled diethyl ether (containing 50 μg of C_{13} internal standard), 0.1 mL of 4 N H_2SO_4 and 2.5 g of granular anhydrous sodium sulfate were added to the sample and the mixture was shaken for 2 hours. After adding 5 mL of hexane the sample was centrifuged (5000 g) for 5 min at room temperature. The sample was then eluted through a 10 mm i.d. glass column containing 1 g of deactivated neutral alumina. The column was washed twice with hexane/diethyl ether (1:1). The column containing alumina with absorbed FFA was dried under vacuum for 1 min. The dried alumina was transferred to a 16 x 150 mm caped glass tube. One mL of isopropyl ether containing 6% formic acid was added and mixed with the alumina. The tube was centrifuged for 5 min at room temperature and the solvent containing the FFA was transferred to a 2 mL sealed vial.

Gas chromatography

Duplicate 1 μL injections were made into a Hewlett-Packard (Palo Alto, CA) Model 5880A gas chromatograph (GC). The GC was equipped with a flame ionization detector and a GC Terminal (Level Four) integrator. The helium carrier

gas flow rate was 30 mL/min. The hydrogen and air were maintained at flow rates of 28 mL/min and 400 mL/min, respectively. The injection and detector ports temperature were maintained at 230 C. The GC column oven was programed at an initial temperature of 100 C, increased at a rate of 15 C/min to 220 C and held at the final temperature of 220 C for 14 min. The FFA were separated on a 15 m x 0.53 mm "Nukol" bonded phase acidic capillary column (Supelco, Inc., Bellefonte, PA). Each FFA was identified by matching retention times with a standard mixture of free fatty acids. All quantitative analyses were derived by relating the peak area of individual FFA to the peak area of the internal standard, tridecanoic acid (C_{13}).

Data analysis

The FFA analysis and sensory data were analyzed using Pearson's correlation coefficients, stepwise regression, stepwise discriminant analysis, discriminant analysis and canonical discriminant analysis. The statistical techniques PROC CORR, PROC DISCRIM, PROC STEPWISE, PROC STEPDISC and PROC CANDISC were applied with Statistical Analysis Systems (SAS Institute Inc.) software programs.

RESULTS AND DISCUSSION

Cheddar Cheese Composition and Near Infrared Spectroscopy

The number of samples used in the calibration set, mean values, standard deviations and ranges for fat, moisture and protein are shown in Table 2. The percent fat, moisture and protein listed in Table 2 were determined by the Babcock, vacuum oven and Kjeldahl procedures (reference methods). The results were similar to the results obtained by Kwak (1988) and were within the standard identity specifications for commercial Cheddar cheese (USDA, 1960).

The calibration correlation coefficients (r) between NIRS spectral data and the chemical methods were .805, .594 and .830 for moisture, fat and protein, respectively. The results were lower than those reported by Frank and Birth (1982) which were .850, .955 and .965 for moisture, fat and protein, respectively. The SEC (standard error of the calibration group) were .624, .882 and .514 for moisture, fat and protein, respectively. Fat gave slightly higher SEC when compared to moisture and protein. The calibration equation should have a high correlation coefficient (r) and a low standard error of calibration (SEC). The SEC is the standard deviation of the individual approximation errors and measures how well the spectral data matches the calibration data. The data for the calibration set is summarized in Table 3.

The wavelengths (terms) selected for each constituent are also reported in Table 3. The protein spectral data was manipulated with the first derivative math

Table 2. Composition of Cheddar cheese as determined by standard laboratory methods.

Constituent	n	Mean[a]	SD	Range
Fat	39	32.81	1.26	28.83 - 36.00
Moisture	39	36.71	1.47	34.26 - 42.27
Protein	39	24.34	0.99	21.26 - 26.45

[a]Mean of 39 Cheddar cheeses; all samples were analyzed in triplicate.

Table 3. Calibration data for near infrared reflectance prediction of Cheddar cheese composition.

Constituent	Calibration Set			
	n	WL[a] (nm)	SEC[b] (%)	r[c]
Fat	38	2238 2300	.887	.587
Moisture	36	2212 2160	.624	.805
Protein	37	2158	.514	.830

[a]Wavelengths selected (nm).
[b]Standard error of the calibration group.
[c]Correlation coefficient of calibration.

treatment which separated overlapping absorption bands and corrected for baseline shift. The moisture and fat spectral data were treated with the second derivative math treatment. The second derivative math treatment separates overlapping absorption bands and corrects for baseline shift, but to a greater extent than the first derivative. The math treatments enables the achievement of higher correlation coefficients (r) and lower standard error of calibration (SEC) for the spectral data.

The statistical program reported outliers for all the component regression equations. Reference data and spectral data for samples 15, 27, and 33 were removed from the moisture equation, sample 33 was removed from the fat equation and samples 27 and 39 were removed from the protein equation. The removal of the data improved the r and SEC values for the component equations.

Once the calibration equation was designed, a validation set was evaluated. Normally, a validation set compares NIRS measurements and reference method measurements on a new set of samples, but due to the limited number of samples, the same calibration samples were used for the validation set.

A comparison between the reference and NIRS determinations for Cheddar cheese composition are presented in Table 4. The means and standard deviations for the reference method analyses and NIRS measurements are also reported in Table 4. The correlation coefficients for the reference values versus the NIRS values for moisture, fat and protein were .817, .616 and .835. The correlation coefficients were significant at $p \leq 0.05$. The standard errors of prediction (SEP) were .598,

Table 4. Comparison of statistical values between chemical analysis and NIRS measurements.

Constituent	n	Mean (%)	SD	r [a]	r^{2} [b]	SEP[c] (%)
Fat	38	32.79[d] 32.79[e]	1.103 .671	.616	.380	.852
Moisture	36	36.70[d] 36.69[e]	1.061 .865	.817	.668	.598
Protein	37	24.41[d] 24.45[e]	.938 .775	.835	.698	.500

[a]Correlation coefficient between chemical analysis and NIRS measurements.
[b]Proportion of explained variance.
[c]Standard error of prediction.
[d]Mean for chemical analysis values.
[e]Mean for NIRS values.

.852 and .500 for moisture, fat and protein, respectively. The SEP measures the standard error between the reference values and the NIRS values. A large SEP percent indicates a narrow degree of error between the NIRS readings and the reference data.

The correlation coefficients for the fat calibration equation as well as correlation coefficient of validation for the comparison of the Babcock values versus NIRS values for fat were low. The low correlation coefficients for fat may have been related to the Babcock method which was used for fat determination. Although the precision of the Babcock fat values was above 97 %, the accuracy of the values may have been inhibited by the method. In the Babcock procedure the percent fat was determined by reading 0.1 % graduations on the neck of the Babcock bottles; therefore, accuracy beyond one tenth of a percent fat was very difficult.

The correlation coefficients for all constituents were lower than published values (Frank and Birth, 1982). The NIRS measures physical parameters while the reference methods employ physical and chemical parameters for the measurement of constituents. The AOAC method for determination of moisture includes moisture and volatile compounds removed from the sample, whereas the NIRS measures the physical energy emitted from the excitement of the molecular bonds in water. The Kjeldahl procedure does not determine protein, but the total nitrogen content, which is not perfectly correlated to the molecular bonding measured by NIRS.

The accuracy and precision of the NIRS can be affected by the method of packing the sample cell, homogeneity of the sample, and particle size of the sample. For all constituents the small sample set most likely resulted in a relatively poor calibration equation. A minimum of 40 to 50 samples which properly represent the population range for the constituent are needed for a calibration set. The maximum number of samples available for this calibration equation was 39; therefore, the level of correlation between the reference and NIRS values was limited.

Sensory Analysis

Mean scores for the aged flavor, flavor defects and body and texture defects are summarized in Table 5. The aged flavor score mean for the mild cheeses was higher than the aged flavor score mean for the medium samples, indicating that the taste panel may have had difficulty distinguishing between the mild and medium samples.

Figure 2 graphically depicts a comparison of the manufacturers labeled age versus the age as perceived by the taste panel. In Figure 2 the regression lines for age as perceived by the taste panel and labeled age of the cheeses intersects in the range of medium to sharp age. The greatest difference between the regression lines can be seen in the mild range and a considerable difference can be seen in the extra-sharp range of the samples. The correlation (r) coefficient between the taste panel responses and labeled age of the samples was .775 ($p \leq 0.01$).

Table 5. A summary of sensory analysis on flavor and body/texture characteristics of commercial Cheddar cheese.

	Characteristic	Age			
		Mild[a]	Medium[b]	Sharp[c]	X-Sharp[d]
Flavor	Aged Flavor[e]	2.91	2.71	4.06	4.44
	Acid[f]	2.37	2.30	2.66	2.33
	Bitter[f]	1.91	1.89	2.30	2.23
	Fermented/Fruity[f]	1.00	1.02	1.02	1.08
	Flat/Lacks Flavor[f]	1.05	1.05	1.02	1.00
	Rancid[f]	1.06	1.09	1.12	1.51
	Sulfide[f]	1.03	1.00	1.15	1.44
	Unclean[f]	1.00	1.04	1.07	1.19
	Whey Taint[f]	1.13	1.11	1.07	1.00
	Other[f]	1.00	1.07	1.04	1.16
Body and Texture	Corky[f]	1.00	1.02	1.01	1.00
	Crumbly[f]	1.07	1.22	1.03	1.00
	Curdy[f]	1.01	1.04	1.01	1.00
	Gassy[f]	1.00	1.00	1.01	1.00
	Mealy[f]	1.10	1.14	1.07	1.04
	Open[f]	1.56	1.32	1.76	1.75
	Pasty[f]	1.04	1.05	1.17	1.19
	Short[f]	1.24	1.12	1.95	2.50
	Weak[f]	1.29	1.16	1.08	1.00

[a]Values are the mean of eight taste panel responses for eleven mild Cheddar cheeses.
[b]Values are the mean of eight taste panel responses for seven medium Cheddar cheeses.
[c]Values are the mean of eight taste panel responses for fifteen sharp Cheddar cheeses.
[d]Values are the mean of eight taste panel responses for six extra-sharp Cheddar cheeses.
[e]A score of 1 = young, 2 = mild, 3 = medium, 4 = sharp and 5 = extra-sharp.
[f]Score for presence of defects; 1 = none, 2 = slight, 3 = moderate, 4 = definite and 5 = extreme defect.

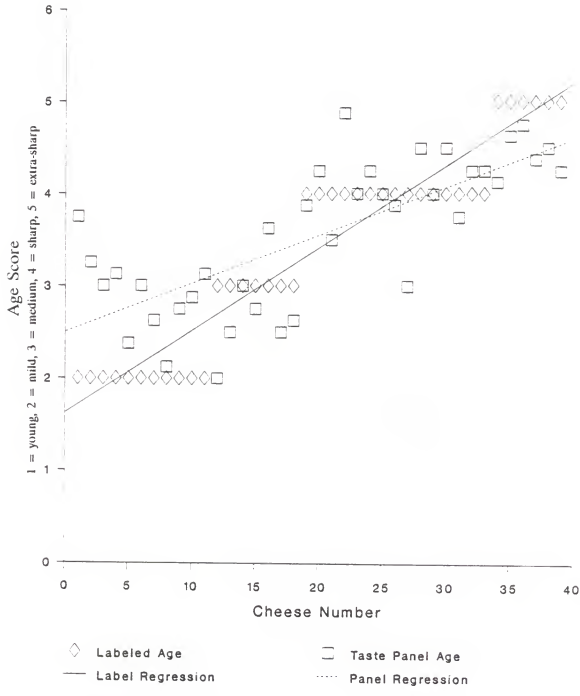

Figure 2. Comparison of taste panel mean response versus labeled age of commercial Cheddar cheese.

FFA Analysis

The mean concentration (ppm) and standard deviation for the individual FFA in cheeses of different ages can be found in Table 6. The concentration of individual FFA were lower than the results obtained by Lin and Jeon (1987) and Marsili (1985), who employed approximately the same isolation technique.

In Cheddar cheeses of various ages the mean concentrations of individual FFA followed the same trend that Marsili (1985) and Woo *et al.* (1984) observed for Cheddar cheeses of various ages. Generally, the concentration of FFA increase as the age of the cheese increases. This trend is most evident in the short-chain FFA (C_4 - C_8) and can be seen more easily in Figure 3. The trend of increasing concentration of FFA as age of the cheese increases was less apparent in the long-chain FFA (Figure 4). The mild cheeses had the lowest concentration of FFA, while the extra-sharp cheeses usually had the highest concentrations of FFA. However, the means for C_8 and longer FFA in the sharp samples concentrations were not higher than the mean concentrations of C_8 and longer FFA in the medium age samples.

The concentrations of FFA were lower than the those found by Marsili (1985), Lin and Jeon (1987) and Deeth *et al.* (1983). The cheeses used in this study were from several manufacturers; consequently the quality and season of the cheese milk, the starter and contaminant bacteria and the ripening conditions might have

Table 6. The mean concentration of individual free fatty acids found in various ages of commercial Cheddar cheese.

	Free Fatty Acid Concentration (ppm)							
	Mild		Medium		Sharp		X-Sharp	
FFA	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C_4	8.54	4.01	11.29	4.58	19.39	18.92	42.85	25.38
C_6	5.98	2.24	7.70	3.71	11.63	10.13	21.52	12.03
C_8	7.85	3.03	11.11	7.68	11.03	7.81	14.02	8.52
C_{10}	60.67	10.73	62.10	6.09	46.58	5.96	84.96	6.89
C_{12}	41.02	10.03	48.37	13.65	43.91	17.67	57.09	25.81
C_{14}	92.17	28.53	108.57	29.23	106.20	43.73	126.85	66.19
C_{16}	128.96	71.36	133.81	81.39	127.87	73.84	137.95	88.84
$C_{18:0}$	40.18	12.52	53.22	22.21	43.12	15.82	101.32	13.52
$C_{18:1}$	146.20	56.26	196.39	129.54	155.65	67.52	162.40	106.29.

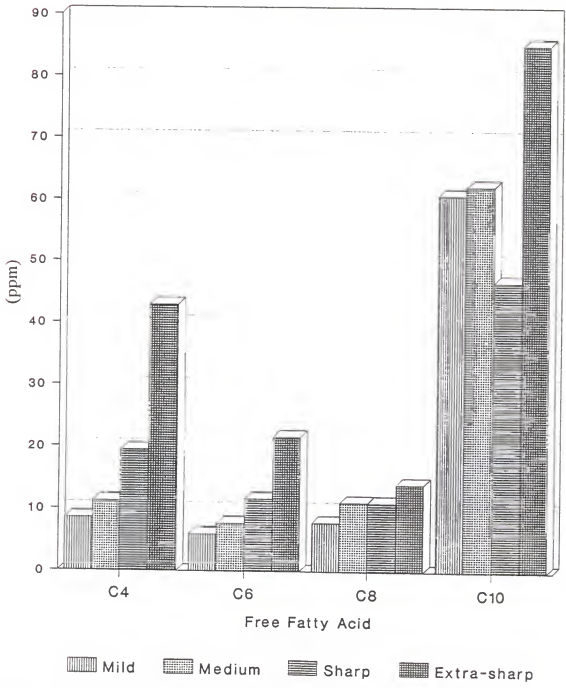

Figure 3. Mean concentration of short-chain free fatty acids by age.

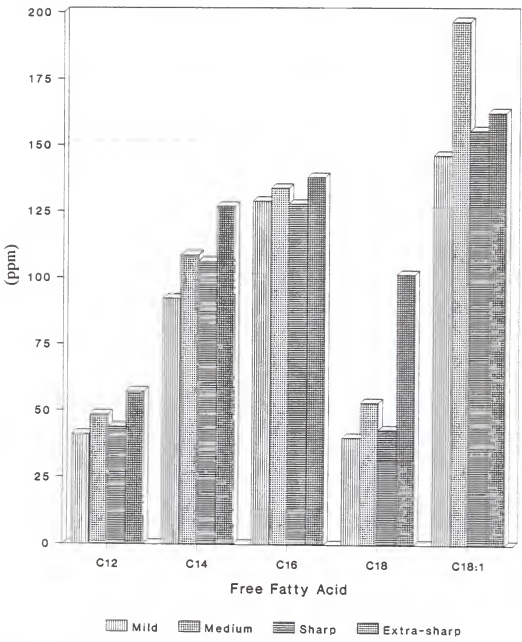

Figure 4. Mean concentration of long-chain free fatty acids by age.

accounted for the individual FFA variability. These factors can all ultimately affect the FFA concentration in Cheddar cheese (Deeth and Fitz-Gerald, 1976).

Free Fatty Acids and Cheese Flavor

Stepwise regression was conducted relating FFA concentration to the mean taste panel scores using the backward elimination technique. In the backward stepwise regression the FFA showing the least correlation to the taste panel score for age were eliminated until all variables were significant at $p = .05$. The regression equation which predicts an expected taste panel age score based on the concentration of C_6, C_{16} and C_{18} was:

$$\text{Predicted panel score} = 4.301 + .071(\text{ppm } C_6) + .008(\text{ppm } C_{16}) - .054(\text{ppm } C_{18}).$$

The multiple correlation coefficient (r) for the regression equation was .805. The r value indicates how well an observed score relates to a score given by the regression equation. The concentration of C_4 could also be a good indicator of panel score for age, because the correlation coefficient between the concentration C_6 and the concentration of C_4 was .956, $p \leq .05$ (Table 7).

Backward stepwise discriminant analysis was used to define age categories and to classify the cheese into age groups based on their FFA concentrations. The backward elimination started with all the FFA in the model and progressively

Table 7. The Pearson correlation coefficients between age as perceived by taste panel, labeled age and concentration of individual free fatty acids.

	Label[a]	Panel[b]	C_4	C_6	C_8	C_{10}	C_{12}	C_{14}	C_{16}	$C_{18:0}$	$C_{18:1}$
Label[a]	1.000										
Panel[b]	.775*	1.000									
C_4	.541*	.594*	1.000								
C_6	.509*	.579*	.956*	1.000							
C_8	.277	.351*	.703*	.850*	1.000						
C_{10}	.034	.018	.258	.242	.157	1.000					
C_{12}	.229	.250	.684*	.773*	.864*	.212	1.000				
C_{14}	.240	.283	.650*	.754*	.872*	.152	.967*	1.000			
C_{16}	.022	.080	.457*	.531*	.705*	-.042	.875*	.884*	1.000		
C_{18}	.254	.205	.399*	.480*	.566*	.050	.606*	.630*	.898*	1.000	
$C_{18:1}$	.028	.048	.456*	.499*	.667*	.160	.695*	.676*	.871*	.078	1.000

[a]Age as labeled by manufacturer.
[b]Age as perceived by the taste panel.
*Significant at $p \leq .05$

removed the FFA which contributed least to the age classifications. The stepwise discriminant analysis indicated that a combination of C_6, C_{16} and C_{18} concentrations classified ($p \leq .05$) the cheese samples into age categories.

A canonical function based on the concentration of C_6, C_{16} and C_{18} was derived using canonical discriminant analysis and only one canonical function was required to summarize the C_6, C_{16} and C_{18} concentrations. The canonical function derived from the canonical discriminant analysis permitted the calculation of a canonical score. The canonical scores for all the samples were calculated using the following equation:

$$\text{Canonical Score} = -.156(\text{ppm } C_6) - .023(\text{ppm } C_{16}) + .150(\text{ppm } C_{18}).$$

The canonical function was used to compute canonical scores for each sample and discriminant analysis was then used to classify the samples into age groups on the basis of their canonical scores. A canonical score less than 1.116 placed the sample into the extra-sharp category, a canonical score greater than 1.116 and less than 2.149 placed the sample into the sharp category, a canonical score greater than 2.149 and less than 3.448 placed the sample into the mild category and a canonical score greater than 3.448 placed the sample into the medium age category. Table 8 displays the percent of cheese samples classified into age categories by discriminant analysis of their canonical scores.

Table 8. Percentage of Cheddar cheeses classified into particular age groups on the basis of their computed canonical scores.

	Percent Classified into Age Categories			
Labeled Age	Mild	Medium	Sharp	Extra-Sharp
Mild	83.3	0.0	16.7	0.0
Medium	16.7	83.3	0.0	0.0
Sharp	18.2	0.0	54.5	27.3
Extra-Sharp	20.0	0.0	0.0	80.0

Stepwise regression was performed relating the panel acid defect score to the concentration of FFA. The backward elimination procedure was used and all FFA were eliminated except for C_8 and C_{10}. The regression equation for predicting acid was :

$$\text{Predicted Acid} = 2.297 + .032(\text{ppm } C_8) - .003(\text{ppm } C_{10}).$$

The multiple correlation coefficient (r) for this regression equation was .673 ($p \leq .05$). Other FFA concentration which could possibly be good indicators of acid were C_{12} and C_{14} because they showed high correlations with C_8 (Table 7).

Backward stepwise regression was also carried out on all other flavor defects. Stepwise regression removed all FFA concentrations from the equation below the significance level of 0.05 ($p \leq .05$), therefore none of the FFA concentrations were considered to contribute to the equation at the selected significance level. The results of the stepwise regression on the remaining flavor defects were not surprising, because all cheeses used in the study were of good quality. The absence of correlation between the remaining defects and the concentration of FFA was supported by Ohren and Tuckey (1969), who observed excessive FFA levels, especially the C_{10}, C_{12} and C_{14}, in poor quality Cheddar cheeses. The cheese they evaluated had fermented, unclean and rancid flavors.

In addition stepwise regression indicated that the concentrations of individual FFA obtained in the study were not related to body and texture defects. Fredrick and Dulley (1984); and de Jong (1977) reported strong correlations between proteolysis and cheese texture; therefore, the lack of correlation between FFA concentration and body & texture was expected.

CONCLUSIONS

Composition Analysis by Near Infrared Reflectance Spectroscopy

Although the correlations coefficients were significant between values for NIRS and reference methods, they need improvement. A larger calibration set should increase the correlation coefficient for the calibration. Larger calibration sets would have maximum spectral variability and would result in a maximum number of terms used in the calibration equation.

Near infrared spectroscopy (NIRS) could be used for rapid determination of fat, moisture and protein. Cheese composition analysis by the NIRS will greatly decrease the time for analysis and will require less sample. Although some initial reference determinations are required for proper calibration, the need for costly and hazardous chemicals will be greatly reduced.

Free Fatty Acids Influence on Cheddar Cheese Flavor

Gas chromatographic separation of FFA and statistical analysis enabled the classification of Cheddar cheese into age categories using the concentration of FFA. Stepwise regression selected the C_6, C_{16} and C_{18} FFA concentrations as the best indicators of the age scores given by the taste panel. The correlation coefficient between the taste panel and the concentrations of C_6, C_{16} and C_{18} was .805 ($p \leq .05$). Stepwise discriminant analysis selected the C_6, C_{16} and C_{18} FFA as the FFA which most efficiently placed the cheese samples into age categories. Canonical

discriminant analysis was used to summarize the age characteristics of C_6, C_{16} and C_{18} FFA into a canonical function. The canonical scores for the samples allowed the categorization of the cheeses into age groups. Eighty-three percent of the mild samples, 83 percent of the medium samples, 55 percent of the sharp samples and 80 percent of the extra-sharp samples were classified correctly by their canonical scores.

The C_8 and C_{10} FFA concentrations were selected by stepwise regression as the best indicators of the acid defect. A regression equation which could be used to predict the acid score for a Cheddar cheese was developed utilizing the fatty acid concentrations and sensory scores . All other Cheddar cheese flavor defects and body & texture defects were not related to the concentration of FFA, individually or in combination.

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Appendix

Appendix 1. Mean percent fat and standard deviation for percent fat as determined by the Babcock procedure and the predicted NIRS values for commercial Cheddar cheese samples.

Sample	% Fat	S.D.	NIR value
1	32.58	0.14	31.89
2	32.92	0.14	33.20
3	32.83	0.29	32.29
4	32.75	0.25	32.74
5	33.17	0.29	33.39
6	30.50	0.50	32.28
7	32.00	1.32	33.04
8	31.25	0.43	32.96
9	31.33	0.29	32.32
10	31.83	0.14	32.19
11	32.12	0.13	32.34
12	31.97	0.15	31.63
13	34.37	0.23	33.41
14	33.67	0.29	32.62
15	30.87	0.32	31.36
16	32.43	0.31	32.36
17	34.40	0.17	33.38
18	33.47	0.45	33.25
19	32.25	0.25	33.78
20	34.60	0.36	32.74
21	33.83	0.29	33.18
22	32.17	0.58	32.23
23	32.57	0.12	32.58
24	32.07	0.60	33.01
25	36.00	0.50	34.54
26	31.83	0.29	32.81
27	32.17	0.29	33.04
28	32.93	0.12	33.01
29	32.77	0.25	32.57
30	33.70	0.17	32.96
31	33.77	0.40	32.45
32	33.83	0.29	33.08
33	28.83	0.29	31.55
34	32.63	0.29	33.31
35	31.77	0.25	31.25
36	33.50	0.50	32.89
37	32.73	0.92	32.45
38	33.57	0.40	33.92
39	32.73	0.25	33.39

Appendix 2. Mean and standard deviation for percent moisture as determined by the vacuum oven procedure and the predicted NIRS values for commercial Cheddar cheese samples.

Sample	% Moisture	S.D.	NIR value
1	36.59	0.11	37.58
2	35.19	0.14	36.31
3	36.38	0.15	36.29
4	37.77	0.07	37.52
5	36.27	0.21	36.52
6	36.79	0.13	36.12
7	37.16	0.08	37.34
8	36.27	0.03	35.64
9	39.09	0.20	38.02
10	35.82	0.20	35.66
11	37.01	0.04	36.27
12	37.70	0.09	36.65
13	36.28	0.12	36.92
14	35.56	1.92	35.65
15	39.32	0.06	38.20
16	35.97	0.06	36.07
17	36.08	0.16	36.98
18	36.67	0.04	35.94
19	37.05	0.05	36.63
20	34.50	0.41	35.68
21	38.05	0.01	37.68
22	36.39	0.01	36.31
23	37.90	0.02	37.46
24	36.72	0.02	35.78
25	34.26	0.21	35.27
26	38.69	0.19	38.64
27	39.53	0.10	38.32
28	36.20	0.03	36.56
29	36.83	0.39	37.15
30	38.10	0.05	37.77
31	36.23	0.63	36.32
32	35.66	0.61	36.03
33	42.27	0.18	39.32
34	36.76	0.20	36.48
35	38.06	0.11	38.57
36	36.57	0.17	37.22
37	37.99	1.01	37.80
38	36.26	0.44	35.90
39	36.40	0.11	36.15

Appendix 3. Mean and standard deviation for percent protein as determined by the Kjeldahl procedure and the predicted NIRS values for commercial Cheddar cheese samples.

Sample	% Protein	S.D.	NIR value
1	24.91	0.29	24.58
2	24.87	0.34	24.63
3	24.17	0.41	25.16
4	24.25	0.03	23.70
5	26.16	0.46	25.12
6	25.75	0.19	25.10
7	24.13	0.19	24.18
8	25.97	0.19	25.58
9	23.47	0.30	23.67
10	26.45	0.17	25.75
11	24.32	0.55	24.59
12	24.63	0.72	24.76
13	24.19	0.22	24.10
14	25.41	0.26	25.60
15	23.78	0.10	23.55
16	25.36	0.04	25.78
17	23.57	0.45	23.78
18	23.85	0.33	25.04
19	24.46	0.29	24.11
20	25.13	0.68	25.27
21	23.85	0.32	23.86
22	25.21	0.14	24.71
23	23.56	0.43	24.01
24	24.87	0.64	25.01
25	24.42	0.31	25.40
26	23.24	0.32	23.26
27	21.96	0.82	23.26
28	25.44	0.37	24.71
29	24.03	0.42	23.87
30	23.55	0.47	23.21
31	25.16	0.04	24.57
32	25.34	0.75	24.81
33	22.24	0.29	22.87
34	24.33	0.06	24.50
35	23.33	0.03	23.37
36	23.57	0.17	24.03
37	23.71	0.04	23.48
38	24.30	0.15	24.85
39	24.44	0.29	24.17

Appendix 4. A summary of the labeled age and the mean taste panel response for the age of commercial Cheddar cheese samples.

Sample	Labeled Age*	Taste Panel Age*
1	2	3.75
2	2	3.25
3	2	3.00
4	2	3.13
5	2	2.38
6	2	3.00
7	2	2.63
8	2	2.13
9	2	2.75
10	2	2.88
11	2	3.13
12	3	2.00
13	3	2.50
14	3	3.00
15	3	2.75
16	3	3.63
17	3	2.50
18	3	2.65
19	4	3.88
20	4	4.25
21	4	3.50
22	4	4.88
23	4	4.00
24	4	4.25
25	4	4.00
26	4	3.88
27	4	3.00
28	4	4.50
29	4	4.00
30	4	4.50
31	4	3.75
32	4	4.25
33	4	4.25
34	5	4.13
35	5	4.63
36	5	4.75
37	5	4.38
38	5	4.50
39	5	4.25

*2 = mild, 3 = medium, 4 = sharp, 5 = medium

Appendix 5. The concentration of individual FFA found in Cheddar cheeses of various ages.

Sample	Age	FFA (ppm)[a]								
		C_4	C_6	C_8	C_{10}	C_{12}	C_{14}	C_{16}	$C_{18:0}$	$C_{18:1}$
1	2	7.44	6.98	10.83	38.96	47.81	112.41	146.66	49.18	160.66
2	2	6.63	4.91	5.19	21.10	40.49	69.08	124.42	46.56	129.17
3	2	14.40	8.42	8.20	383.15	43.24	89.31	66.98	41.11	148.26
4	2	12.07	7.01	9.97	31.64	41.90	95.60	30.99	30.66	152.98
5	2	7.92	5.88	8.52	31.07	47.98	101.96	180.89	52.63	167.67
6	2	15.95	10.51	13.89	47.42	60.36	154.68	223.79	55.91	282.71
7	2	8.64	6.53	9.37	32.61	45.81	112.50	--	51.43	179.68
8	2	4.52	3.32	5.21	20.83	29.71	59.53	--	18.85	97.72
9	2	4.89	4.84	5.90	23.28	37.61	90.16	--	41.53	123.52
10	2	3.62	2.92	4.24	17.16	23.91	55.96	--	23.56	75.65
11	2	7.91	4.47	4.98	20.13	32.32	72.73	--	30.55	90.83
12	3	6.92	4.54	5.42	19.77	40.53	78.16	73.53	44.96	122.07
13	3	11.44	7.64	9.03	35.29	48.56	115.23	126.21	59.52	210.86
14	3	9.99	8.17	10.58	34.97	52.85	111.70	130.71	55.21	139.58
15	3	21.08	15.60	27.96	79.32	72.59	157.59	279.42	96.50	478.61
16	3	10.53	6.34	9.56	36.67	49.78	118.34	148.59	53.47	176.56
17	3	8.47	5.05	5.90	193.78	27.27	68.03	44.37	27.81	100.49
18	3	10.63	6.56	9.31	34.91	46.98	110.94	--	35.04	146.59
19	4	31.89	17.11	14.23	254.49	58.63	146.17	169.17	54.37	207.38
20	4	18.64	10.07	8.86	27.71	43.39	112.29	160.16	44.98	200.59
21	4	10.24	6.21	7.70	27.46	42.20	94.61	106.51	44.40	132.12
22	4	74.47	32.46	20.26	51.69	65.32	149.71	166.58	54.89	251.75
23	4	3.71	2.30	2.38	9.71	16.35	34.58	45.61	19.56	53.25
24	4	31.40	18.78	20.44	57.72	72.37	172.24	238.73	62.22	215.12
25	4	22.90	15.53	14.11	42.13	66.16	162.85	237.65	71.61	260.13
26	4	8.80	5.70	5.95	21.25	37.81	76.18	67.41	28.14	84.88
27	4	6.45	4.71	6.37	25.39	39.13	89.81	--	33.65	107.41
28	4	40.31	34.22	31.43	64.08	64.33	160.04	131.95	57.39	206.41
29	4	11.16	6.71	7.58	28.20	34.20	96.39	--	49.47	176.81
30	4	8.67	5.45	6.07	20.72	26.69	65.12	47.28	26.61	96.53
31	4	8.11	6.62	9.64	33.86	42.50	113.76	--	48.48	171.31
32	4	6.36	4.35	5.81	21.33	28.61	68.86	--	29.04	96.96
33	4	7.74	4.30	4.75	14.99	20.99	50.36	35.48	22.00	74.06
34	5	53.03	22.41	10.63	147.45	49.64	102.23	116.88	56.35	198.69
35	5	37.51	19.59	10.72	189.33	58.79	101.88	108.14	42.79	159.75
36	5	83.94	39.78	22.47	63.92	75.36	176.78	251.86	79.00	307.58
37	5	24.08	13.47	12.13	41.26	64.48	150.12	193.12	48.66	227.05
38	5	47.68	28.74	25.60	59.89	83.76	208.22	--	373.42	52.44
39	5	10.83	5.11	2.55	7.89	10.48	21.86	19.04	7.70	28.90

[a]Values are the mean of duplicate determinations.

THE INFLUENCE OF FREE FATTY ACIDS ON AGE AND FLAVOR CHARACTERISTICS OF COMMERCIAL CHEDDAR CHEESE AND COMPOSITION ANALYSIS USING NEAR INFRARED REFLECTANCE SPECTROSCOPY

by

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ABSTRACT

Thirty-nine commercial Cheddar cheeses of various ages and from several manufacturers were analyzed by a gas chromatographic technique for free fatty acids (FFA) content. The cheese samples were also evaluated by a trained taste panel for age characteristics, flavor defects and body and texture defects. Stepwise regression analysis was utilized to develop a regression equation which predicted the taste panel age score using the C_6, C_{16} and C_{18} FFA concentrations (ppm). The multiple correlation coefficient for the panel age regression equation was .805 ($p \leq .05$).

Stepwise discriminant analysis was utilized with the FFA concentrations to classify the samples into age categories. The C_6, C_{16} and C_{18} FFA concentrations were selected as the parameters which best reflect the age categories. Canonical discriminant analysis was used to develop a canonical function and a canonical score was calculated for each sample. Prediction ranges were developed to allow the estimation of a samples age. The samples age was predicted on the basis of the canonical score calculated by the canonical function using the concentration of the C_6, C_{16} and C_{18} FFA. Results of discriminant analysis performed on the canonical scores for the samples indicated that 83 percent of the mild, 83 percent of the medium samples, 55 percent of the sharp and 80 percent of the extra-sharp samples were classified correctly.

A regression equation was developed and related the panel acid score to the FFA concentrations of the samples. A combination of C_8 and C_{10} FFA concentrations was selected as the best indicator of the taste panel acid score. The

multiple correlation coefficient for the acidity regression equation was .673 ($p \leq .05$). The concentrations of C_{12} and C_{14} FFA could also be good indicators of Cheddar cheese acid defect because they were highly correlated to C_8.

Composition analysis of commercial cheese was determined using the Babcock procedure for fat, vacuum oven procedure for moisture (AOAC), and the semi-micro-Kjeldahl procedure for protein (AOAC). The results were compared to composition analysis by near infrared reflectance spectroscopy (NIRS). The correlation coefficients (r) between the chemical and NIRS determination were .616, .817 and .835 for fat, moisture and protein, respectively. The correlation coefficients were not as high as expected, but could be improved with a larger and more detailed calibration set. The NIRS exhibited the capability of rapid composition analysis although the correlation coefficients require improvement before the expected precision can be obtained.