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Study of Ripening Characteristics of Full-Fat and Low-Fat Cheddar Cheese Using Fourier Transform Infrared Spectroscopy and Texture Analyzer

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STUDY OF RIPENING CHARACTERISTICS OF FULL-FAT AND LOW-FAT CHEDDAR CHEESE USING FOURIER TRANSFORM INFRARED SPECTROSCOPY AND TEXTURE ANALYZER

by

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ABSTRACT

Study of Ripening Characteristics of Full-Fat and Low-Fat Cheddar Cheese Using Fourier Transform Infrared Spectroscopy and Texture Analyzer

by

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A suitable microtome sampling technique was used to sample cheese for analysis using FTIR spectroscopy. Well-separated fat- and protein-related bands were obtained in the spectra of Cheddar and Mozzarella cheese samples using this method. The absorbance intensity of the spectra was proportional to the thickness of the sample. The intensity of absorbance at fat- and protein-related bands increased with an increase in the fat and protein content in the sample. Strong and well-separated bands at 1744, 1450, 1240, 1170, and 1115 cm^{-1} arising mainly from fat content were observed using this method. Bands observed at 1650 and 1540 cm^{-1} were attributed to the protein present in the cheese. Bands at 1615-1639, 1640-1648, 1650-1658, and 1660-1688 cm^{-1} corresponding to β-sheet, random coil, helix, and the turns/sheet portion of the secondary structure were observed in the range of the amide I band.

Characteristics of spectra for full-fat (FFCC) and reduced-fat Cheddar cheese (RFCC) during ripening were investigated. The absorbance of bands at 1116-1240 from C-C, C-O, C-N stretch, 1461 cm^{-1} from C-N bend (scissoring), 1744 cm^{-1} from ester carbonyl groups (fat A), 2850-2930 cm^{-1} from C-H stretch (fat B), 1650 and 1540 cm^{-1} from protein amide I and II varied during cheese aging. Bands at 1116 and 1240 cm^{-1} arising from C-O, C-N, and C-C stretch changed slightly during cheese aging. A correlation coefficient of 0.97 for bands between 1744 and 1167 cm^{-1} arising from fat, and that of 0.93 at 1650 and 1540 cm^{-1} arising from protein, respectively, showed that one of these fat or protein groups was highly related to the other. A correlation coefficient of greater than 0.80 among the bands of fat and protein groups indicated a strong interaction in those bands. Correlation of ripening time and absorbance at bands corresponding to each function group was analyzed. A ripening index model was obtained by correlating ripening time with predominant reactive group absorbance peaks. An R^2 of 0.83 and 0.59 was obtained for full-fat and reduced-fat Cheddar cheese, respectively.

Texture development and its correlation with FTIR spectra data for FFCC and RFCC during aging were also studied. RFCC had a higher value of hardness, gumminess, and chewiness than its full-fat counterpart. The values decreased during the early stages of ripening and then increased with time. The change in hardness, adhesiveness, and springiness was expressed as a function of the change in absorbance of the FTIR spectra using multiple regression analysis. An R^2 value of 0.67, 0.54, and 0.75 was obtained for full-fat Cheddar cheese, and a value of 0.51, 0.59, and 0.54 was obtained for reduced-fat Cheddar cheese for the respective texture parameters. (131 pages)

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CHAPTER 1

INTRODUCTION

PROBLEM STATEMENTS

Consumer concerns about the levels of fat and cholesterol in diets have brought about the development of a large array of low-fat and nonfat dairy foods. However, the manufacture of acceptable high quality low-fat or nonfat cheese is probably the most technologically challenging of all dairy food processes.

Cheese is made up of a network of interconnected strands of protein that surround globules of fat. Water, either bound or absorbed, is a major component of all cheeses (Johnson and Chen, 1995). Levels of fat in cheese have a direct impact on acceptability. When the fat was reduced by 25% in Cheddar cheese, it compared well with its full-fat counterpart (Olson and Johnson, 1990). Researchers noted that reduction of fat content by 33% yielded Cheddar-type cheese that was acceptable, but reduction by 50% or greater resulted in cheese of lower flavor and physical properties.

Reduction in the fat levels of Cheddar cheese results in a firm, more elastic, dry, and crumbly cheese with less flavor (Emmons et al., 1980; Lawrence and Gilles, 1987). Johnson and Chen (1995) pointed out that the firmness of cheese is not controlled solely by its composition. The ionic interactions between protein strands influenced by the pH and bound calcium played a major role in the firmness of cheese. However, the most important contributing factor to the firmness of cheese is the level of proteolysis that occurs during ripening. Products of proteolysis, i.e., amino acids and peptides and

especially compounds derived from them, are known to contribute to the flavor of cheese (both desirable and undesirable) as it matures.

The ripening process is catalyzed by enzymes such as proteinase, peptidase, phosphatase, lipase, decarboxylase, transaminase, redoxase, those that decompose amino acids, and enzymes that facilitate respiration and fermentation. The proteolysis of casein was found to be of particular importance in cheese ripening because of the presence of protein in large quantities in cheese (Schormüller, 1968). Casein, particular the α_s-moiety, is hydrolyzed first, whereas β- and ρ-κ-casein are not greatly proteolysed in most bacteria-ripened cheeses (Nauth and Ruffie, 1995). Texture development during aging occurs in two phases (Lawrence and Gilles, 1987). *Phase one* constitutes the first 7-14 days when the rubbery texture of young cheese is rapidly converted to a smoother, more homogenized product as a result of a breakdown of α_{s1}-casein. *Phase two* involves a more gradual change in texture over the months as a result of a continuing breakdown of α_{s1}-casein and other casein.

Proteolysis during ripening affects not only flavor but also texture. Texture or body of cheese is one of the most important functional properties because it is by this property that the consumer determines the identity and quality of a specific variety of cheese (Lawrence and Gilles, 1987). The difference in the texture of different cheese varieties is related to the difference in proportion of the components of cheese, rennet, milk, casein, moisture, lactic acid, sodium chloride, fat, and calcium (Lawrence and Gilles, 1987). But as in most solid materials, the structure of cheese influences texture the most. The solid structure of the cheese is due primarily to the cross linked casein-calcium

phosphate network, entrapped within which is fat and water (Lawrence and Gilles, 1987). The three major components contributing toward the structure of the cheese are casein (protein), moisture, and fat. The distribution and the manner in which they are held in the cheese system determine texture.

Work has been done in the past to relate texture development (Lawrence and Gilles, 1987) and proteolysis (Fox et al., 1994) in cheese during aging. However, very limited work has been done to study the effect of biochemical changes on texture in cheese, and more so in low-fat cheese, during ripening.

Near-infrared (NIR) spectroscopy has been shown to be useful for direct, rapid, and nondestructive quantitative analysis of major components in solid and semisolid foods, and has been applied to analyze fat and moisture in Cheddar cheese. However, this method requires large calibration data sets and correlation methods (Pierce and Wehling, 1994). Fourier transform infrared (FTIR) spectroscopy has been used to analyze food products, such as cheese (McQueen et al., 1995), meat (Dion et al., 1992), fats and oils (van de Voort et al., 1994), butter/margarine (van de Voort et al., 1992a), sweetened condensed milk (van de Voort et al., 1992b), and sugar/juice (Hopkins and Newberry, 1986). Most of the past applications dealt with quantitative analysis, and none dealt with relating biochemical reaction to functional properties. FTIR spectroscopy has also been used to determine the secondary structure of proteins (Surewicz and Mantsch, 1988; Sarver and Krueger, 1991).

The structure of cheese is altered with age as a result of a series of biochemical and microbiological changes that affect the casein network. To manufacture an acceptable

high quality low-fat or nonfat cheese, it is very important to know the biochemical changes and their corresponding effects on functional properties such as texture and flavor. Proper understanding of the mechanism will help in the development of a suitable process that will result in a product that is of appeal to consumers. However, this is difficult since most analytical procedures for measuring biochemical groups and changes in cheese during aging are complex and time consuming. Not much work has been done on studying biochemical changes of functional groups that occur during aging, and their relation to texture development in reduced-fat cheese systems.

OBJECTIVES

The aim of this work was to study the absorbance changes of typical functional groups related to fat and protein in cheese during ripening by using Fourier transform infrared spectroscopy and obtain an index for ripening and a correlation for different textural properties. The specific objectives were to:

1) examine the characteristics of spectra of different fat levels of Cheddar cheeses during ripening;
2) establish a correlation of cheese ripening as a function of absorbance of selective functional groups and develop a ripening index;
3) correlate changes in absorbance intensity of functional groups related to fat and protein in cheese during ripening with changes in texture properties (hardness, adhesiveness, and springiness).

LITERATURE REVIEW

Studies on biochemistry of cheese ripening

Cheese ripening involves numerous reactions in cheese due to the micro flora and constituents. The main causes of cheese ripening are microbiological- and enzyme-induced changes. Among the ripening reactions are the decomposition and re-synthesis of all substances involved in the reaction, such as proteins, peptides, amino acids, carbohydrates, lipids, nucleic acids, organic acids, various carbonyl compounds, growth factors from the groups of vitamins, prosthetic groups of enzymes, and finally, simple decomposition products, such as carbon dioxide and ammonia (Schormüller, 1968).

Schormüller (1968) indicated that the ripening process is affected by the mechanism, the microorganisms used, the reaction products formed, and pH of the cheese block. Substances that change during cheese ripening include nitrogen substances, the carbohydrate components, fat components, acids, cheese flavor compounds, vitamins, carbon dioxide fixation, and acetate utilization compounds.

Nauth and Ruffie (1995) compared the microbiological and biochemical profile of stored samples of 50% reduced, one-third reduced, and no-fat cheese with that of full-fat Cheddar cheese during 30 to 60 days of ripening. They concluded that, for a full-fat Cheddar cheese, α_s-casein, ρ-κ-casein, and, to some extent, β-casein were proteolyzed yielding greater concentration of 11-20 K and 9-11 K molecular weight fractions in cheese; free fatty acids, C_2 through C_{12}, showed a gradual increase in concentration in 60 days of ripening; a flavor volatile, such as ethanol, diacetyl, and pentanone, decreased in concentration at 60 days compared to 30 days; added starter bacterial populations

declined, but adventitious organisms/adjuncts might grow during ripening; and the total calcium retained in reduced-fat cheese was higher by 30% compared to its full-fat counterpart.

From the above-mentioned work, obviously the chemical groups and their changes are different for different cheese and maturation. The various reactions occurring during ripening will affect the concentration of main substances in cheese, which in turn will influence the spectra-elements and the FTIR absorption spectrum of cheese.

The absorption at 1744 cm^{-1} (fat A), characteristic of ester carbonyl groups (R(CO)OR/OH) could be used as an indicator of fat in the system. A decrease in the number of ester groups caused by enzymatic hydrolysis is accompanied by a decrease in absorbance of this band, but an increase in bands was observed around 2850-2930 cm^{-1} (fat B) and 1563 cm^{-1} (protein) for milk samples (van de Voort et al., 1987). Suggested reasons for the increase in signal at fat B were hydrogen bonding between water and released fatty acids, dimerization of free fatty acids, and absorption by the CH_2 groups on the glycerol part of the molecule, all of which absorb at this wavelength (Biggs et al., 1987). The increase in the protein signal is attributed to an absorption of the carboxylate anion of soluble free fatty acids at 1563 cm^{-1} (Sjaunja, 1984). Fat A wavelength measures the number of ester linkages present, which effectively measures the molecular concentration of fat. Changes in the mean molecular weight of fat caused by changes in fatty acid composition will cause a variation between the chemical and infrared methods (van de Voort, 1980). On the other hand, decarboxylation and oxidative deamination of

amino acids can produce short chain fatty acids (Reiter et al., 1966). This increase in fatty acids will in turn influence the fat A or B absorbance.

Applications of infrared spectroscopy in food analysis

Goulden (1961) first used infrared radiation to measure fat, protein, and lactose in milk. The measurement of fat was based on absorbance at 1724 cm^{-1} (fat A wavelength) by ester carbonyl groups of fat molecules. Protein measurement was based on absorbance at 1538 cm^{-1} by peptide bonds of protein molecules, and lactose measurement was based on absorbance at 1042 cm^{-1} by hydroxyl groups of lactose molecules.

Near-infrared spectroscopy can be used to analyze moisture, fat, protein, and total solids in cheese (Pierce and Wehling, 1994; Rodriguez-Otero et al., 1995). Rodriguez-Otero et al. (1995) used near-infrared reflectance spectroscopy to analyze fat, protein, and total solids in cheese without any sample treatment. A set of 92 samples of cows' milk cheese was used for an instrument calibration by principal components analysis and modified partial least-square regression. The following statistical values were obtained: standard error of calibration (SEC) of 0.388 and squared correlation coefficient (R^2) of 0.99 for fat; SEC of 0.397 and R^2 of 0.98 for protein, and SEC of 0.412 and R^2 of 0.99 for total solids. To validate the calibration, an independent set of 25 cheese samples of the same type was used. Standard errors of calibration were 0.47, 0.50, and 0.61 for fat, protein, and total solids, respectively, and R^2 for the regression model obtained was 0.98 for the three components.

By combining attenuated total reflectance (ATR) and mid-infrared spectroscopy (MIRS) with the statistical multidimensional technique, Safar et al. (1994) obtained relevant information from mid-infrared spectra of lipid-rich food products. Wavelength assignments for typical functional groups in fatty acids were made for standard fatty acids. Absorption band around 1745 cm^{-1}, due to carbonyl group, 2853 and 2954 cm^{-1} due to C-H stretch, 3005 and 960 cm^{-1} due to C=C bonds, 1160 cm^{-1} due to C-O bonds, 3450 and 1640 cm^{-1} due to O-H bonds were observed. Water strongly absorbs in the region of 3600-3000 cm^{-1} and at 1650 cm^{-1} in butter and margarine, allowing one to rapidly differentiate the foods as a function of their water content. Principal component analysis was used to emphasize the difference between spectra and to rapidly classify 27 commercial samples of oils, butter, and margarine.

In contrast to NIR, Fourier transform infrared spectroscopy (FTIR) has much to offer the analyst because specific bands may be assigned to specific chemical entities. Statistical correlation methods are not always necessary, but they are not excluded and may be required in very complicated mixtures (Belton et al., 1987). This technique has been widely used to determine fat, moisture, and protein in butter (van de Voort et al., 1992a), meat (Dion et al., 1992), sweetened condensed milk (van de Voort et al., 1992b), and other high-fat products (van de Voort et al., 1993). It has also been used to monitor the oxidation of edible oil (van de Voort et al., 1994) and to determine the level of *trans*-unsaturation in fat (Ulberth and Haider, 1992).

The use of FTIR for qualitative measurements in cheese is very limited (McQueen et al., 1995) primarily due to the difficulty in sampling procedure or due to the nonavailability of a standard procedure (Perkins, 1993).

FTIR related study and analysis

Sarver and Krueger (1991) devised an infrared (IR) method to determine the secondary structure of proteins in solution using the amide I region of the spectrum. The infrared data matrix was constructed from the normalized Fourier transform infrared spectra from 1700 to 1600 cm^{-1} of 17 commercially available proteins. The secondary structure matrix was constructed from the X-ray data of the seventeen proteins with secondary structure elements of helix, β-sheet, β-turn, and other (random) elements. By analyzing the proteins of the CD and IR databases, they concluded that the peak positions of 1660, 1653, 1650, and 1634 cm^{-1} are frequencies assigned for β-turn, helix, other, and β-sheet conformations, respectively, which are consistent with reported literature values of 1666-1688, 1655-1657, 1650, and 1627-1642 cm^{-1}, respectively (Surewicz and Mantsch, 1988).

Belton et al. (1988) studied the components of fat, protein, and sugar in confectionery products by using Fourier transform infrared spectroscopy coupled with photo acoustic and attenuated total reflectance detection methods. They concluded that peaks at 1744, 1477-1400, 1240 and 1195-1129 cm^{-1}, could be from an ester carbonyl group, C-H bend, and C-O stretching of fat, respectively; peaks at 1650 and 1540 cm^{-1} are from protein, and that at 1128-952 cm^{-1} is from sugars.

Water is strongly absorbed between 3600 and 3000 cm^{-1} and at 1650 cm^{-1} in fat-rich foods (Belton et al., 1988; van de Voort et al., 1992a). Principal component analysis was used to emphasize the differences between spectra and to rapidly classify each sample (van de Voort et al., 1992a).

Usually, wavelength assignments for typical functional groups in fatty acids are absorption bands around 1745 cm^{-1} (fat A) for ester carbonyl groups (R(CO)OR/OH), 2930 and 2853 cm^{-1} (fat B) for C-H stretch in methylene groups, and 1160 cm^{-1} for C-O bonds of lipid (Silverstein et al., 1991).

The wavelengths of protein-related functional groups are asymmetric and symmetric N-H stretch, and hydrogen-bonded primary amide around 3350 and 3170 cm^{-1}; the wavelength of C=O stretch in amide band which overlaps with that of N-H bend in 1640 cm^{-1} (amide I); and the C-N stretch assigned to a wavelength of 1425 cm^{-1} (Silverstein et al., 1991).

Instrumental analysis of texture

By studying the textural properties of 11 different varieties of cheese, Chen et al. (1979) reported that fat did not contribute significantly to the variations in the textural attributes of cheese. Hence, fat was considered to play a minor role in the classification of cheese (Fox, 1987). However, other studies (Emmons et al., 1980) showed that reduced fat cheese was harder and more elastic, described as rubbery, than the full-fat cheese. This is considered a major impediment in the manufacture of low-fat cheese and was discussed in the review of low-fat cheese (Jameson, 1990). The rubbery texture of low-fat cheese is attributed to the increase in a structural matrix per unit cross-sectional area.

Instrumental analyses of textural properties of cheese are important for quality control and its use as a tool for researchers, to study cheese structure (Tunick et al., 1990). All instrumental tests can be divided into three classes (Konstance and Holsinger, 1992), fundamental tests, empirical tests, and imitative tests. Fundamental tests measure the basic rheological properties, force-compression, creep, stress relaxation, and shear. Empirical tests are food specific and are developed to give good correlation with sensory evaluations. Puncture, shear, and extrusion are a few examples in this category. Imitative tests imitate the action of the mouth on the food. Texture profile analysis is an example.

Fundamental tests have been found to correlate poorly with the sensory evaluation of the textural properties of foods. This can be attributed to the fact that in foods the structure does not break under normal conditions of use as in the case of engineering applications but is smashed into thousands of little pieces under the influence of limited forces available in the mouth (Bourne, 1978). In the case of imitative tests the textural properties are assessed by a device which imitates the action of the mouth and thus is more reflective of the way in which humans perceive the sensory property.

Imitative tests gained importance with the major breakthrough of the development of a device to perform a texture profile analysis (TPA), the General Food Texturometer (Szczesniak et al., 1963). Analysis of a force-time curve led to the extraction of seven texture parameters. The texture parameters (Szczesniak et al., 1975) are as follows: Fracturability (originally called brittleness) is the force at the first significant break at the curve; hardness is defined as the peak force at the first compression cycle ("first bite"); cohesiveness is the ratio of the positive force area during the second compression to that

during the first compression; adhesiveness constitutes the negative force area from the first bite, representing the work required to pull the compressing plunger away from the sample; springiness (originally called elasticity) is the height that the food recovers during the time that elapses between the end of the first bite, and the start of the second bite; gumminess is the products of hardness and cohesiveness; chewiness is the product of hardness, cohesiveness, and springiness.

TPA has been widely used by the researchers to study the textural properties of food including cheese (Trepanier et al., 1991). Most consumers do not completely understand the meaning of textural properties defined by hardness, fracturability, cohesiveness, gumminess, chewiness, adhesiveness, and springiness to describe the mechanical characteristics. Hence, popular nomenclature, better appreciated by the consumer, is used for sensory evaluations. The relationship between the textural parameters and popular nomenclature is outlined in Table 1.1 (Szczesniak et al., 1963).

Table 1.1--Relationship between texture parameters and popular nomenclature

Primary parameter	Secondary parameter	Popular term
hardness		soft, firm, hard
cohesiveness	fracturability	crumby, crunchy, brittle
	chewiness	tender, chewy, tough
	gumminess	short, meaty, pasty, gummy
elasticity		plastic, elastic
adhesiveness		sticky, tacky, gooey

Although considerable work has been published to support the rubbery nature of low-fat cheese, not much work has been done to study and compare the difference in texture properties of reduced-fat cheese and full-fat cheese. Also, there is no work to elucidate the relationship between textural properties with the functional groups in cheese during aging.

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CHAPTER 2

A SAMPLING METHOD FOR CHEESE ANALYSIS USING FTIR SPECTROSCOPY

ABSTRACT

A microtome sampling technique was used to sample cheese for analysis using FTIR spectroscopy. Well-separated fat- and protein-related bands, such as fat A at 1744 cm^{-1}, fat B from 2850 to 2930 cm^{-1}, amide I at 1620-1690 cm^{-1}, and amide II at 1500-1580 cm^{-1}, were obtained in the spectra of Cheddar and Mozzarella cheese samples using this method. The absorbance of spectra was proportional to the thickness of sample. Moisture affected the spectra of cheese samples, but this was eliminated by equilibrating sample in a sample holder for at least 10 min. The absorbancy intensity of fat and protein related bands increased with an increase in fat and protein contents. Bands at 1615-1639, 1640-1648, 1650-1658, and 1660-1688 cm^{-1} corresponding to β-sheet, random coil, helix, and the turns/sheet portion of secondary structure were observed in the range of the amide I band. This technique could be used to study the chemical groups and to rapidly determine fat and protein in cheese samples.

INTRODUCTION

Fourier transform infrared (FTIR) spectroscopy is now a widely used technique in analytical and research laboratories (Belton et al., 1987). Most analytical procedures for measuring moisture, fat, and protein in cheese are time-consuming and destructive to the

sample (Pierce and Wehling, 1994). Rapid analysis techniques for fat, protein, and total solids content determination in milk by infrared absorption spectroscopy investigated in the past have been widely adopted in the dairy industry (McGann, 1978). Infrared milk analysis is an approved AOAC method (Biggs, 1972).

However, infrared absorption spectroscopy is only suitable for liquid samples; consequently, cheese and other solid products must be blended and homogenized before analysis (Biggs, 1979). Cheese is difficult to analyze spectroscopically using traditional methods because of its textural characteristics (McQueen et al., 1995). The authors studied the use of optothermal near-infrared (OPT-NIR) spectroscopy and Fourier transform mid-infrared attenuated total reflection (FTIR-ATR) spectroscopy to obtain protein, fat, and moisture contents from 24 cheese samples. For OPT-NIR, the sapphire surface containing the sample is mounted on the top of a small self-contained optothermal instrument in which the heat generated when light is absorbed by a sample is measured as a function of temperature change. In that experiment, the sample of cheese was applied to the ATR element of the FTIR spectrometer and spread across the entire surface using a soft spatula. The authors concluded that the optothermal method is superior to the FTIR-ATR method for determining the fat, protein, and moisture contents of cheeses because of the fact that the FTIR-ATR method was affected by sample nonhomogeneity and fat adsorption to the ZnSe ATR crystal.

Most of the past work required an extensive sample preparation process or was applied to general food products. The work presented describes a new simple sampling technique for analyzing cheese using FTIR spectroscopy with the aid of a microtome unit.

Sample spectra for cheese with different fat levels will be provided. A simple technique to study the change in protein structure and other bands related to fat and protein is also provided.

MATERIALS & METHODS

Samples

Different levels of reduced-fat Mozzarella (8.9% and 20.0% fat content) and Cheddar (8.6%, 16.9%, 24.4%, and 32.3% fat content) cheeses were used in the analysis. The cheese samples were made in the Utah State University Dairy Plant (Department of Nutrition and Food Sciences) through the Western Dairy Center.

Sample preparation

Cheese samples for FTIR analysis were prepared using the following procedure: Small pieces of sample (15 mm in height and 15 mm in diameter) were cut from the center of a cheese block and frozen at -80 °C for at least 2 hr. Each frozen sample was then sliced to a thickness of 4, 8, or 16 μm using a IM236 microtome (International Equipment Co., Needham Heights, MA) and then attached to the surface of a silver chloride crystal and placed in the light path of the FTIR spectrometer light beam.

FTIR analysis

Spectra of the sliced frozen film were collected by the Mattson Polaris™ FTIR spectrometer (Mattson Instruments, INC., Madison, WI) equipped with a triglycine sulphate (TGS) detector. The collected spectroscopic data were processed using Mattson

Polaris Icon Software and Bio-Rad Win-IR software. Spectra of samples in the region between 4000 cm^{-1} and 400 cm^{-1} were obtained with a resolution of 1, 4, and 8 cm^{-1} using a scanning frequency of 16, 32, and 64 scans/sample, at 1, 5, 10, and 15 min after they were placed in the light path. The collected spectroscopic data were processed to obtain the area of peak using the peak report option of Bio-Rad Win-IR software (Bio-Rad, Cambridge, MA).

Proximate analysis

The percentage compositions of fat, protein, and moisture were determined using the standard methods outlined in the standard methods for the examination of dairy products (Marshall, 1993). Fat content was determined using the Babcock method (method 15.8d), moisture content by vacuum oven method (method 15.10A), and protein content using the Kjeldahl method (method 15.12A). All samples were tested in triplicate.

RESULTS & DISCUSSION

Optimization of FTIR analysis

The spectra (400 - 4000 cm^{-1} range) of samples collected at resolutions of 1, 4, and 8 cm^{-1} (denoted by symbols a, b, and c, respectively, in Fig. 2.1) indicate that acquisition at a high resolution provides a smoother spectrum, but the signal-to-noise ratio decreases with an increase in resolution. Hence, an intermediate resolution of 4 cm^{-1} was chosen. The effect of scans per sample on spectra at the same resolution (Fig. 2.2) was not significant. Symbols a, b, and c in Fig. 2.2 correspond to the spectra obtained at 16, 32, and 64 scans/sample, respectively. The sampling time per sample increased with

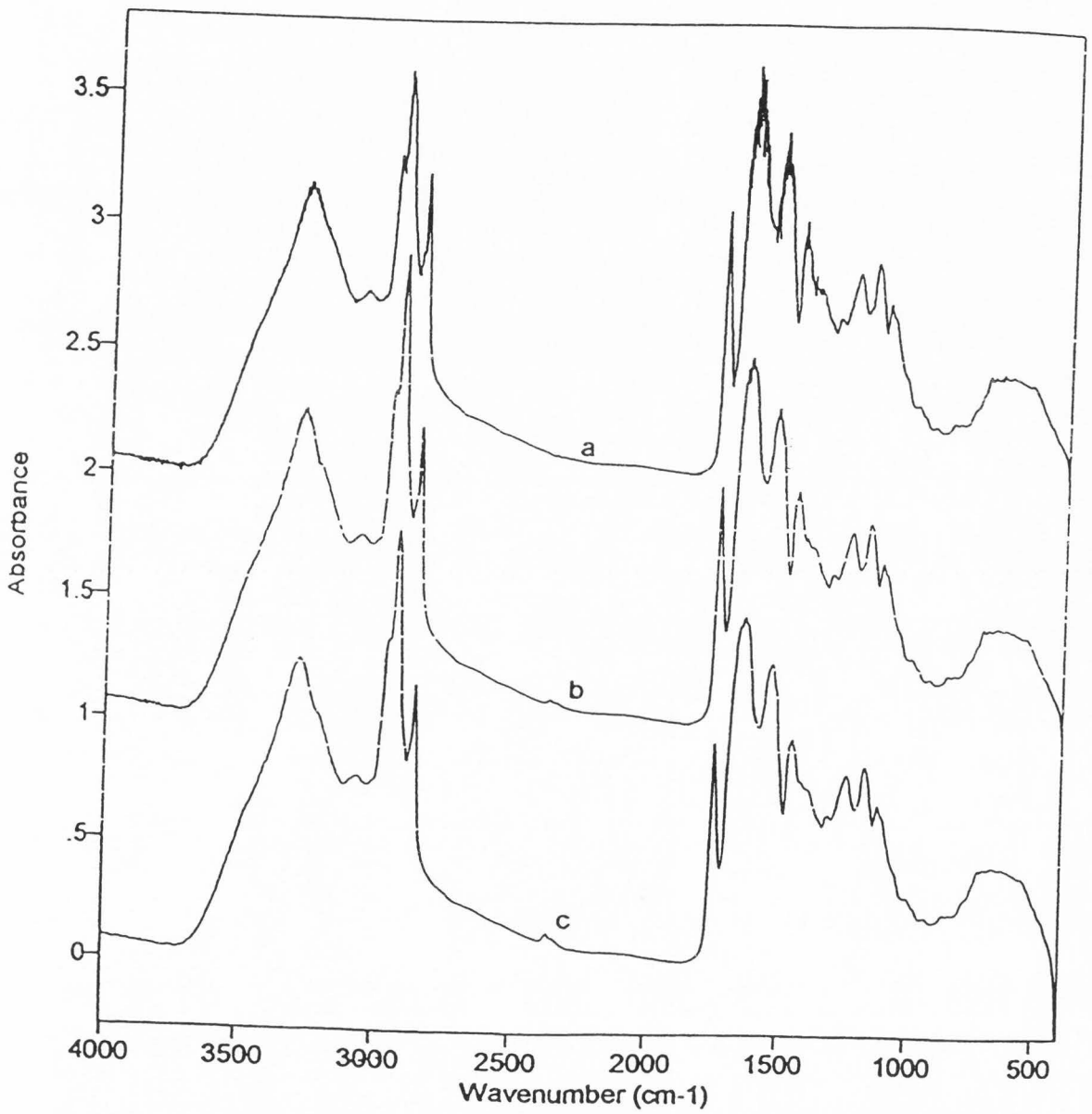

Fig. 2.1--Spectra of 50% RFCCs collected using resolutions of a) 2/cm, b) 4/cm, and c) 8/cm with a sample thickness of 8 μm, a scanning frequency of 32 scans/sample, and 10 min after their exposure in the sample holder.

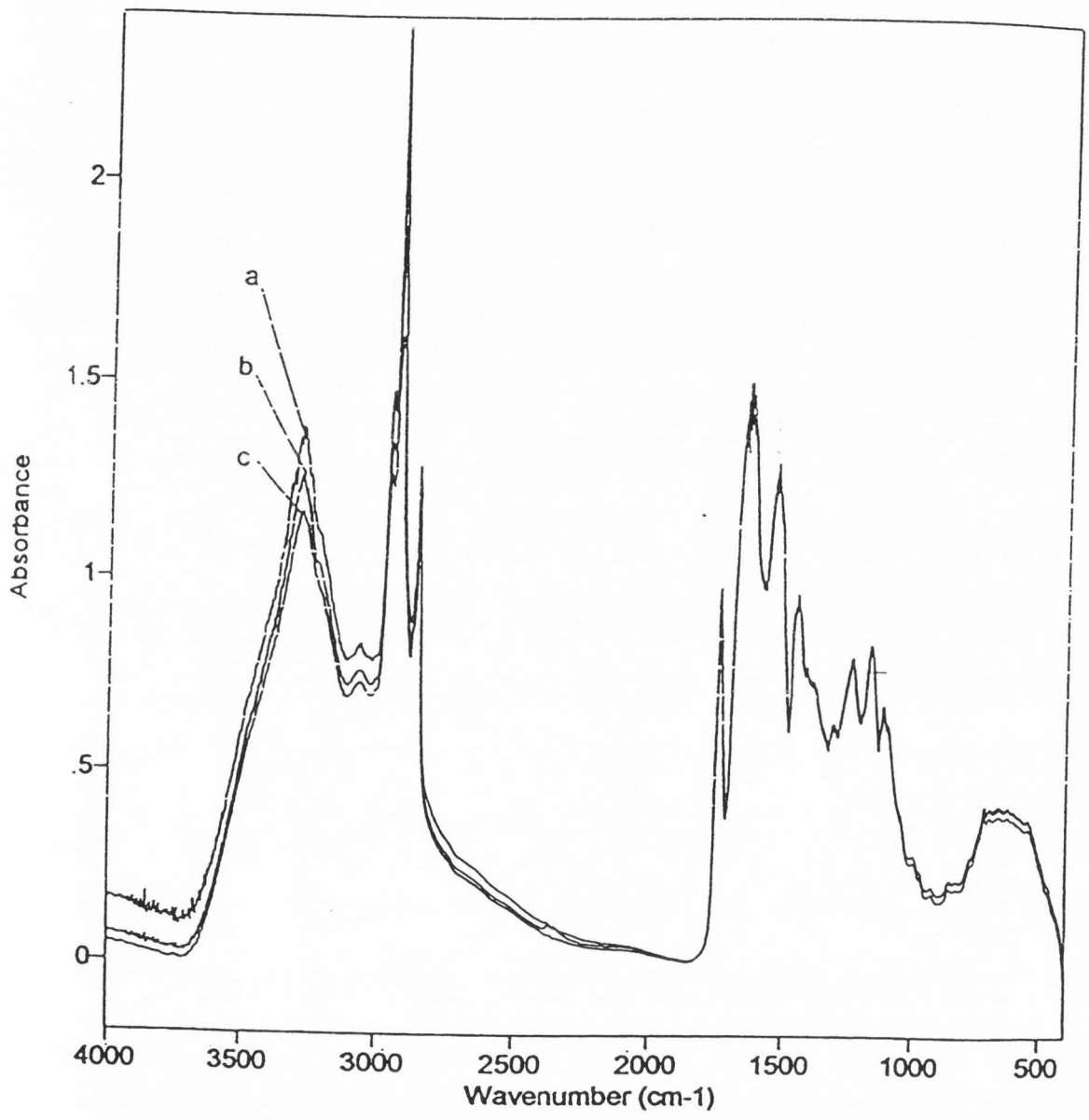

Fig. 2.2--Spectra of 50% RFCCs collected using scanning frequencies (scans/sample) of a) 16, b) 32, and c) 64 with a sample thickness of 8 μm, a resolution of 4/cm, and 10 min after their exposure in the sample holder.

the number of scans for the same resolution and mirror velocity. Thirty-two scans/sample were chosen as it provided a flatter baseline than 16 scans/sample but the same as 64 scans/sample. Spectra of samples with different thicknesses are shown in Fig. 2.3. Thickness of the sample slice can be controlled to be in the range between 2 and 16 μm in 2-μm increments using a microtome. The intensity of spectral absorbance, which is proportional to the sample thickness, is consistent to Beer's Law. Thickness of the sample was kept to a maximum value of 16 μm to accommodate samples from various sample slicing units and to accommodate the nonhomogeneities in the sample.

Moisture effects

Fig. 2.4 shows the spectra acquired 1 min, 10 min, and 20 min after placing Mozzarella cheese sample in the film holder. Liquid water has a very strong, broad band, which is highly absorbing in the 3100 to 3700 cm^{-1} range and a weaker band around 2000 cm^{-1}; another strong band at 1640 cm^{-1} was also observed (Silverstein et al., 1991). At about 800 cm^{-1}, water stops transmitting altogether. The moisture in cheese affected the spectra of microtome-frozen cheese samples by masking or modification of the strong broad bands at 3000 to 3600 cm^{-1} and the amide I band in the range between 1600 and 1700 cm^{-1}. This is consistent with the observation by Safar et al. (1994) whose work indicated that water strongly absorbs in the region of 3000-3600 cm^{-1} and at 1650 cm^{-1}. The moisture bands also affected the multiple N-H bonds (Silverstein et al., 1991) in the 3330-3060 cm^{-1} region. The masking of the spectra was especially predominant during the initial stages, due to the presence of free water. However, this can be eliminated by collecting the spectra 10 min after placing them in the light path; this will minimize the

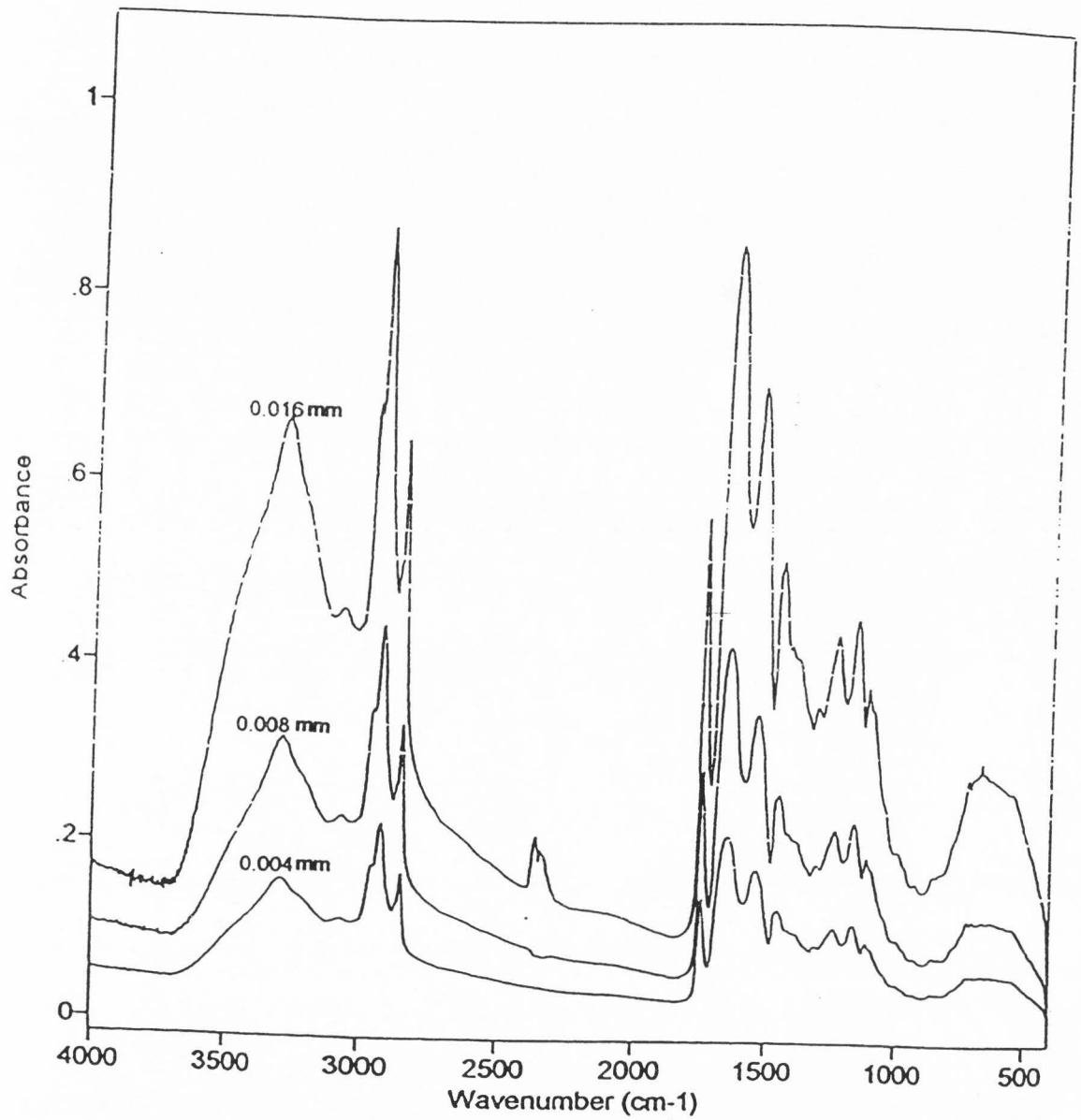

Fig. 2.3--Overlaid spectra of 50% RFCCs collected with sample thicknesses of 0.004, 0.008, and 0.016 mm at a resolution of 4/cm, 32 scans/sample, and 10 min after their exposure in the sample holder.

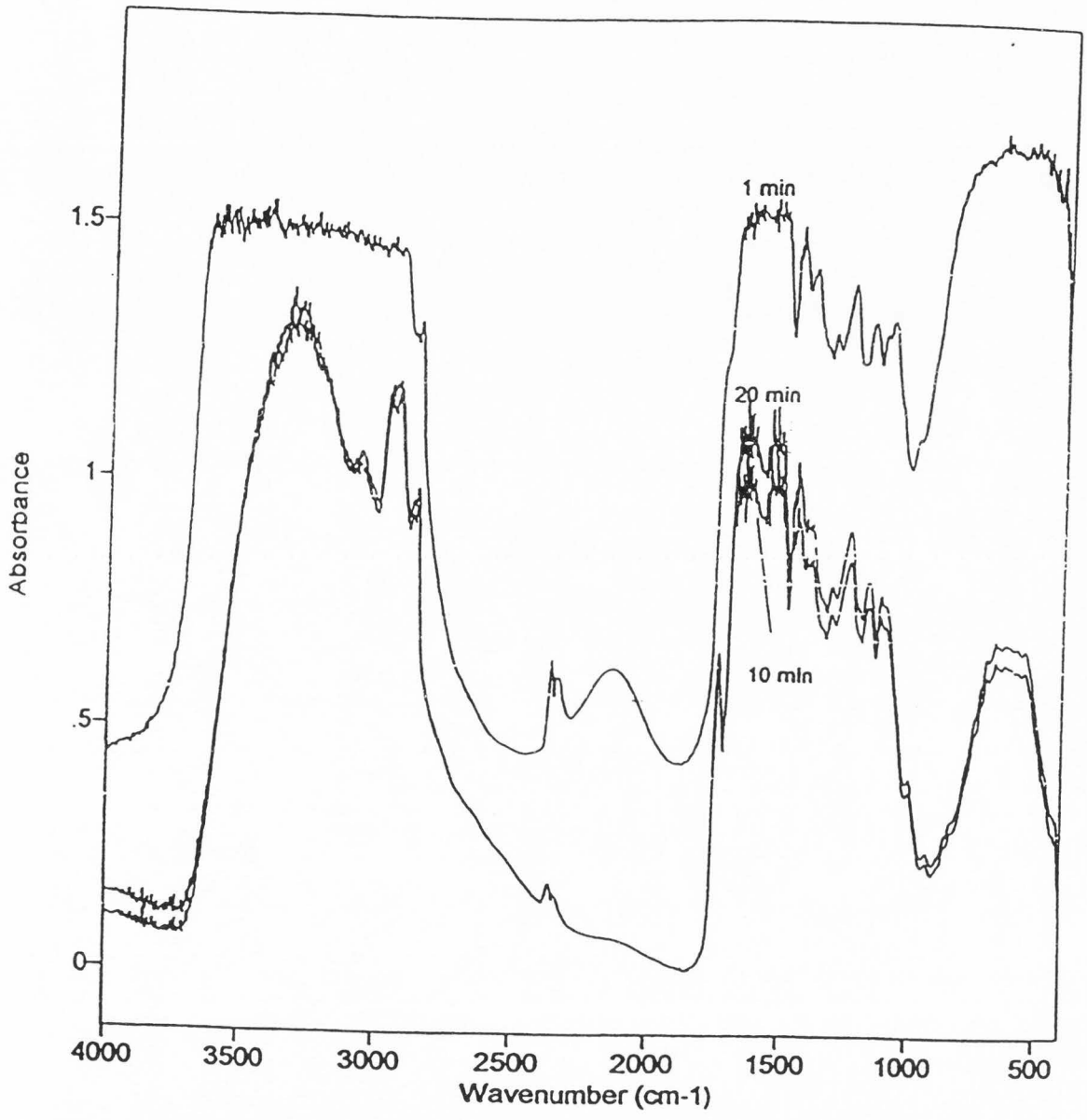

Fig. 2.4--Overlaid spectra of low-fat (9%) Mozzarella cheese acquired at 1, 10, and 20 min after its exposure in the sample holder.

distortion due to moisture variation in the sample. distortion due to moisture variation in the sample.

Sample application

Variations in contact between the sample and silver chloride crystal affect signal strength. For a given sample, a slight gap between the sample and the surface crystal should cause a reduction of signal strength, which may offset the total spectrum if the sample were not attached properly. The effect is more evident with the reduced-fat (hard) cheese than with the full-fat (soft) samples tested. This variation can be minimized by pressing the sample piece with cold wax paper after its placement on the crystal. Sample thickness also affects sample application since it is difficult to place a very thin sample slice on the surface of the crystal. The thinner the sample, the weaker its strength. Hence, in our measurement, a maximum thickness of 16 μm was used to improve sample application.

Sample inhomogeneity

Cheese samples used in experiments are hardly ever perfectly homogeneous using this sampling technique. For wet chemistry quantitative analysis, at least 1 g of sample is required (about 5 g for moisture, 9 g for fat, and 1 g for protein content determination). In our FTIR analysis the sample weight is only about 0.1 mg (at maximum thickness of 16 μm). To obtain appropriate averages for quantitative analysis using this method, several pieces from each cheese sample should be analyzed and the results averaged. From our experience, it is recommended that a minimum of five replications should be used, and

the sampling locations should be smooth and free of void spaces. A hole or a dent in the 16-μm thick sample will affect the absorbance and, hence, the spectra. The total time taken for sampling and spectra acquisition is about 15 min per experiment.

Characteristics of spectra for cheeses

The spectrum of a representative full-fat Cheddar cheese sample is shown in Fig. 2.5. Well-separated bands were observed in the spectrum. Strong bands at 1745, 2950-2800, 1477-1400, 1240, and 1160-1106 cm^{-1} from ester carbonyl groups, C-H stretching, C-H bend, and C-O stretching of fat (Belton et al., 1988; Mendenhall, 1991), respectively, can be observed. Peaks at 1650 and 1536 cm^{-1} corresponding to protein (Belton et al., 1988; Wilson et al., 1988) are also apparent. Strong bands of water in the range between 3600-3100 cm^{-1} and 1640-1650 cm^{-1}, which overlap with protein amide I band at 1620-1690 cm^{-1} (Garland, 1994), are present. Similar findings were observed by Wilson et al. (1988) in bread, van de Voort et al. (1992) in butter, and Safar et al. (1994) in their work on edible oils, butters, and margarine.

Spectra of different levels of Cheddar samples, 75% RFCC (8.6% fat), 50% RFCC (16.9% fat), and FFCC (32.3% fat), and Mozzarella, 9% RFMC and 20% RFMC (8.9% and 20.0% fat), were collected at the same conditions. The absorbance area of fat-related and protein-related peaks for different cheese samples was compared with the fat and protein contents measured by proximate analysis (Table 2.1). Absorbance of fat-related bands decreased with reduction of fat level in cheese as expected. The trend of protein-related bands was opposite to that of fat bands. This was in agreement with the proximate analysis because a reduction in fat constituted a slight increase in protein

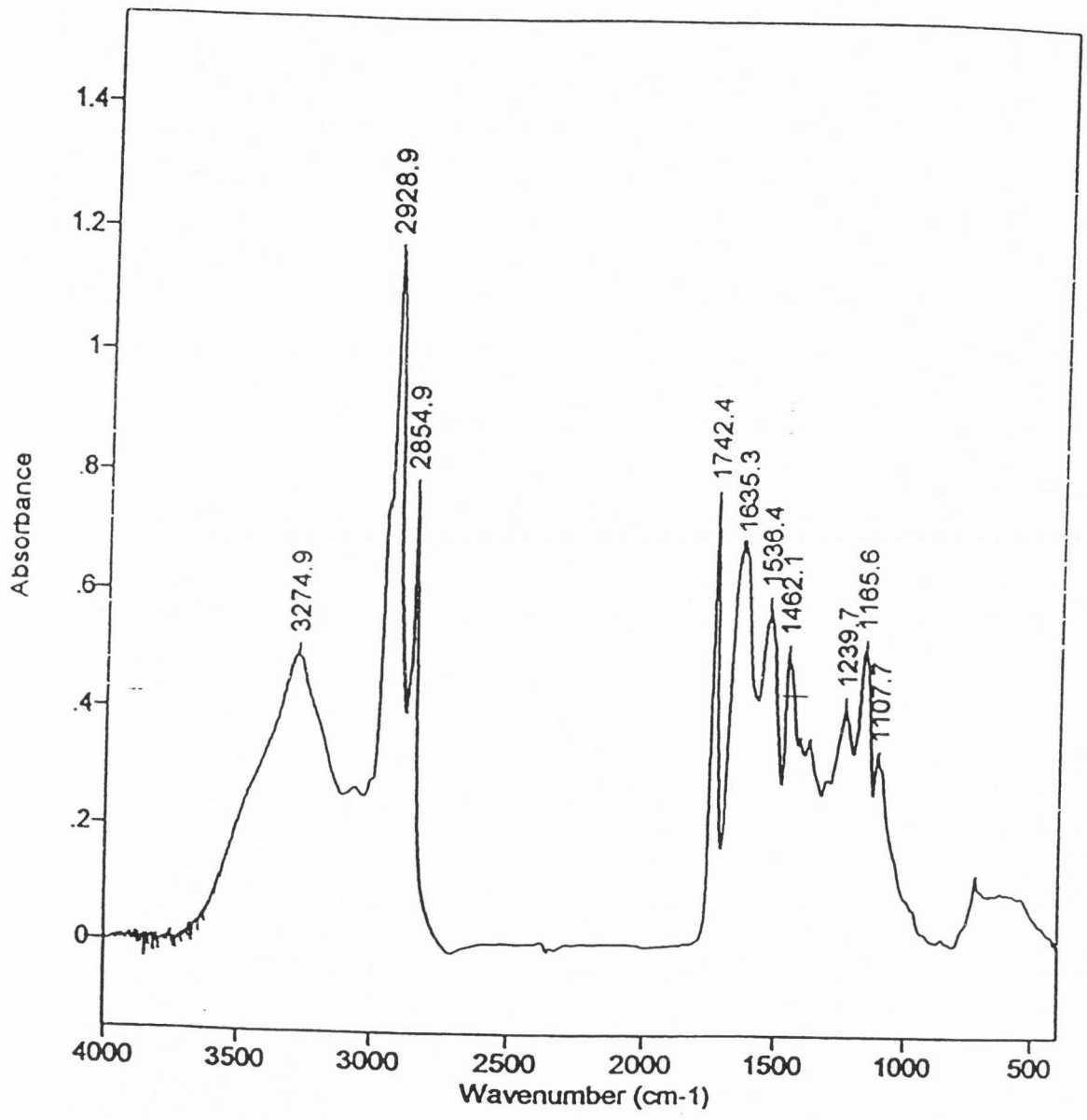

Fig. 2.5--Typical spectrum of FFCC with key bands identified, which was collected at a resolution of 4/cm, 32 scans/sample, and 10 min after its exposure in the sample holder.

Table 2.1--Comparison of data obtained from FTIR analysis and proximate analysis

Sample	Fat content (%) (proximate analysis)	Area of peak from FTIR		Protein content (%) (proximate analysis)	Area of peak from FTIR	
		1744 cm^{-1}	1168 cm^{-1}		1650 cm^{-1}	1540 cm^{-1}
75%RFCC	8.6	7.8	2.9	30.7	41.8	19.75
50%RFCC	16.9	10.8	4.7	26.1	34.5	15.48
FFCC	32.3	15.5	6.8	20.4	22.1	8.15
9%MC	8.9	8.0	3.1	32.7	46.7	24.55
20% MC	20.0	11.3	5.4	27.1	29.9	13.97

content. Mendenhall (1991) reported similar trends for the increase in intensity of protein-related bands at 3030 to 2500 cm^{-1}, 1698 to 1656 cm^{-1}, 1621 to 1038 cm^{-1}, 1017 to 990 cm^{-1}, and 850 to 841 cm^{-1} with the protein contents of milk; all correlation coefficients were greater than 0.90. The fat-related bands at 2994 to 2825 cm^{-1}, 1799 to 1724 cm^{-1}, 1471 to 1451 cm^{-1}, 1284 to 1103 cm^{-1}, and 860 to 859 cm^{-1} also increased with the fat content of milk, with correlation coefficients greater than 0.90.

Frequencies of secondary structure-related bands in Cheddar cheeses

The secondary structure of protein is reflected in the IR spectrum by the absorbance in the amide I region, which is between 1620 and 1690 cm^{-1} and is primarily due to the stretching vibrations of the carbonyl groups (Garland, 1994). The absorbance bands around 1635 cm^{-1} can be associated with the beta-structure, while the bands close to 1653 or 1646 cm^{-1} are associated with the helical portions and random portions of the protein, respectively (Susi and Byler, 1988). Frequencies of peaks in the amide I region, which are related to the secondary structure of casein in all Cheddar cheese samples tested, 75% RFCC (8.6% fat), 50 %RFCC (16.9% fat), 25% RFCC (24.4% fat), and FFCC (32.3% fat), are tabulated (Table 2.2). Frequencies of 1615-1639, 1644-1648, 1650-1655, and 1660-1688 cm^{-1} corresponding to β-sheet, random coil, α-helix, and β-turns/sheet, respectively, are observed for all Cheddar cheese samples.

FTIR spectroscopy coupled with a microtome sampling technique could provide new insights into the secondary structure of casein in a cheese system. Similar techniques have been adopted by Boye et al. (1995) to monitor the change in protein secondary

structure of whey proteins using FTIR spectroscopy. Surewicz and Mantsch (1988) have also discussed the application of infrared spectroscopy for determining the secondary structure of membrane and water-soluble proteins.

The results indicate that there is a tremendous potential to apply this technique to study the changes in chemical groups during cheese ripening and to quantitatively estimate the fat and protein contents in hard or semihard cheeses. FTIR results could be combined with capillary electrophoresis results to study changes in chemical groups and their corresponding compounds.

Table 2.2--Frequencies of secondary structure-related bands and band assignments for Cheddar cheeses

Wavelength (cm^{-1})				Band assignment
75% FFCC	50% FFCC	25% FFCC	FFCC	(tentative[a])
1683		1681		turns
	1672	1672		β-sheet/turns
1667	1667	1667	1667	β-sheet/turns
	1660	1660	1660	turns
1655	1650	1651	1651	α-helix
1648	1645	1644	1644	random coil
1639	1633	1633	1633	β-sheet
1626		1621		β-sheet
1616	1615	1615		β-sheet

[a] (Krimm and Bandekar, 1986; Casal et al., 1988; Susi and Byler, 1988).

CONCLUSION

Well-separated spectra of cheeses were obtained by using microtome sampling technique at a resolution of 4 cm^{-1}, using 32 scans/sample, and a sample thickness of 16 μm. Moisture in the sample was critical for a satisfactory performance using this measurement technique. Repeatable spectra could be obtained after equilibrating the sample for at least 10 min. Sample inhomogeneity and the manner of application are main factors that affect the accuracy and reproducibility of spectra. However, this could be minimized by taking samples from different locations in the cheese and improving the attachment of the sample to the silver chloride crystal. The absorbance intensity of fat- and protein-related bands was proportional to the fat and protein contents in cheese sample. FTIR spectroscopy coupled with the microtome sampling accessory could also be used to monitor changes in the secondary structure of casein protein due to proteolysis during ripening.

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CHAPTER 3

EXAMINATION OF SPECTRAL CHARACTERISTICS OF FULL-FAT AND REDUCED-FAT CHEDDAR CHEESE DURING RIPENING USING FOURIER TRANSFORM INFRARED SPECTROSCOPY

ABSTRACT

The Fourier transform infrared (FTIR) spectra of reduced-fat Cheddar cheese (RFCC) and full-fat Cheddar cheese (FFCC) during aging were examined. Strong and well-separated bands at 1744, 1450, 1240, 1170, and 1115 cm^{-1} arising from fat were observed by using the frozen-microtome samples. Bands at 1650 and 1540 cm^{-1} were attributed to the protein present in the cheese sample. The absorption intensity of protein and fat bands corresponded to the protein and fat content of the samples. Distinct changes in the bands of fat and protein for RFCC and FFCC samples were observed during aging. Both the proteolysis and lipolysis affected the absorbance of fat A band at 1744 cm^{-1}, but lipolysis was more significant than proteolysis for cheese sample after aging for 14 weeks. A change in absorption intensity at wavelengths of 1688 to 1660, 1658 to 1650, 1648 to 1644, and 1633 to 1615 cm^{-1}, assigned to beta-turns/sheet, α-helix, random coil, and β-sheet, respectively, during ripening, is indicative of the change in secondary structural elements due to proteolysis. This technique could be applied for a rapid characterization and determination of the age of cheese undergoing ripening.

INTRODUCTION

While the demand for reduced-fat cheese continues to surge in the US (Dexheimer, 1992), the production of these products still remains a challenge in many respects particularly with regard to flavor, texture, and keeping quality. The significant change in cheese composition due to fat reduction modifies the microenvironment, which in turn modifies the bacterial growth patterns and enzyme activity (Mistry, 1995; Nauth and Ruffie, 1995). The final character of Cheddar cheese depends not only on the initial composition of the product but also on the biochemical and chemical changes that occur during maturation (Banks et al., 1995). The effects of manufacturing process, composition of milk (such as fat level), and the biochemical events that occur during ripening play an important role in the production process. One of the important factors that will help in the process and product development is an understanding of the interaction of different components in the cheese system and how they change during ripening.

Near-infrared (NIR) spectroscopy has shown tremendous potential in applications that involve direct, rapid, and nondestructive quantitification of major components in solid and semisolid foods, and has been applied to analyze fat and moisture in Cheddar cheese (Pierce and Wehling, 1994). However, this method requires a large calibration data and a correlation method. It is difficult to use this method to measure and monitor the biochemical events (glycolysis, lipolysis, and proteolysis) and the secondary catabolic changes (deamination, decarboxylation, beta-oxidation, and even ester formation) that occur during production and ripening.

In contrast to NIR, Fourier transform infrared spectroscopy (FTIR) has much to offer the analyst because specific bands can be assigned to specific chemical entities (D'Esposito and Koenig, 1978). Statistical correlation methods are not always necessary, although they are not excluded and may be required in very complicated mixtures (Belton et al., 1987). This technique has been widely used to determine fat, moisture, and protein in butter (van de Voort et al., 1992a), meat (Dion et al., 1992), sweetened condensed milk (van de Voort et al., 1992b), and other high-fat products (van de Voort et al., 1993). It has also been used to monitor the oxidation of edible oils (van de Voort et al., 1994), and determine the level of *trans*-unsaturation in fats (Ulberth and Haider, 1992).

The use of FTIR for qualitative measurements in cheese is very limited due to the difficulty in sampling procedure or due to the nonavailability of a standard procedure (Pierce and Wehling, 1994). Spectra from wetted systems were obtained using FTIR coupled with attenuated total reflectance (ATR) (Belton et al., 1987). However, a useful calibration graph could not be constructed because the amide I band was obscured by absorption due to water, and in some instances the amide II was superimposed on a sloping baseline and was considerably offset. The effect of moisture also affected the resolution and reproducibility of spectra.

The main objective of this chapter was to investigate the application of FTIR spectroscopy to examine the characteristics of spectra for different fat-level Cheddar cheeses and corresponding changes during their ripening.

MATERIALS & METHODS

Milk and cultures

Skim milk from the Utah State University Dairy Products Laboratory was standardized to 3.6%, 2.7%, 1.8%, and 0.9% with cream using Pearsons equation to produce full-fat and reduced-fat Cheddar cheeses. The culture, C.S.S.® bulk set dairy cultures (Mesophilic lactic acid producing cocci, CTD) from Waterford Foods Inc. (Millville, UT), was used for cheese. The culture was grown in low fat milk (2%) at 30 °C for about 5 hr before being used.

Cheddar cheese manufacturing procedure

Full-fat Cheddar cheese (FFCC) and reduced-fat Cheddar cheese (RFCC) with three levels of fat reduction, 25% RFCC, 50% RFCC, and 75% RFCC, were made in the Gary H. Richardson Dairy Products Laboratory at Utah State University (Logan, UT). The cheeses were made in three separate vats, and samples from all the vats were used in the analysis. The manufacturing procedures of the FFCC and RFCC were slightly different and followed the steps listed in Tables A1 and A2.

Proximate analysis

The percentage compositions of fat, protein, salt, ash, pH, and moisture were determined using methods outlined in the standard methods for the examination of dairy products (Marshall, 1993). Fat content was determined using the Babcock method (method 15.8d), moisture content by vacuum oven method (method 15.10A), and protein content using the Kjeldahl method (method 15.12A). Samples were tested in triplicate.

To determine the non-protein nitrogen (NPN) content, the sample was solubilized in 0.1 M NaOH. Trichloroacetic acid (TCA) (15%) was then added to give a final concentration of 12%. The precipitated protein was removed using Whatman No. 42 filter paper, and the filtrate was analyzed for nitrogen content using the Kjeldahl method.

The proteolysis of casein for full-fat and 75% reduced-fat Cheddar cheese at day 1 and 90 was analyzed using described procedure of capillary electrophoresis (Strickland et al., 1996).

FTIR analysis

Cheese was prepared for FTIR analysis using the same procedure mentioned in Chapter II. The frozen film was then placed on the surface of a silver chloride crystal in the light path of the Mattson Polaris^{TM} FTIR spectrometer (Mattson Instruments, Inc., Madison, WI) equipped with a triglycine sulphate (TGS) detector. The spectra of cheese samples were collected at least 10 min after placing them in the film holder. Spectra of samples in the region of 4000 and 400 cm^{-1} were obtained with a resolution of 4 cm^{-1} and a scanning frequency of 32 scans/sample. The collected spectroscopic data were processed to obtain the area of peaks using the integration option of Bio-Rad Win-IR software (Bio-Rad, Cambridge, MA).

RESULTS & DISCUSSION

Spectra of FFCC and RFCC

The spectra of FFCC and all the RFCC (25%, 50%, and 75%) had a good resolution and signal-to-noise ratio as shown in Fig. 3.1. In the spectra, a number of bands

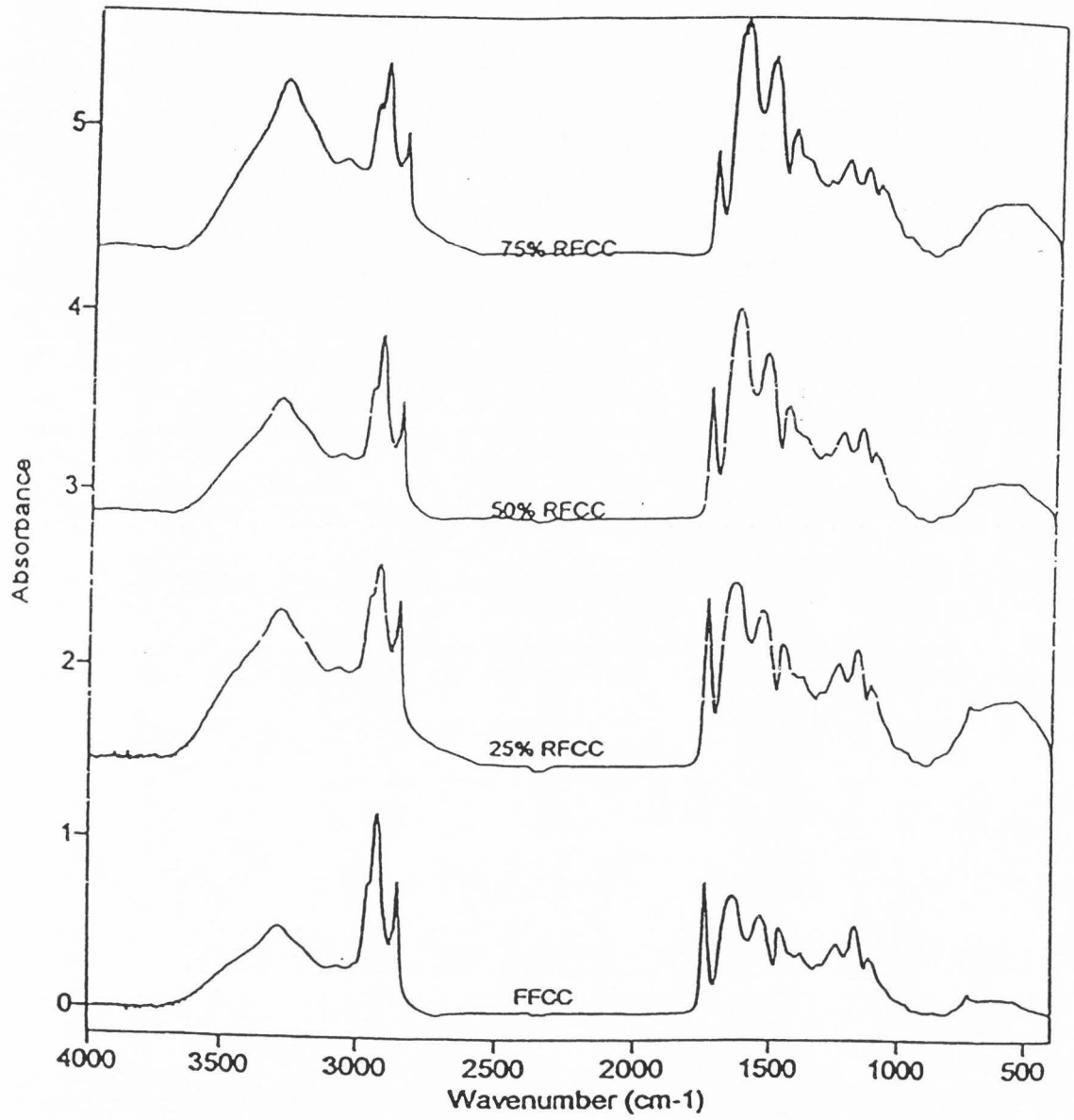

Fig. 3.1--Spectra of 3-months-aged FFCC and 25%, 50%, and 75% RFCCs.

arising from fat were observed, with the strongest for fat A between 1730 to 1765 cm^{-1} arising from ester carbonyl group of various R(CO)OH/R and at 2930 and 2850 cm^{-1} arising from C-H stretch vibration, generally associated with methyl and methylene groups (Silverstein et al., 1991). Similar to the work reported by Belton et al. (1988), fat-related bands occurring at 1477 to 1400 cm^{-1} (C-H bending), 1240 cm^{-1}, and 1170 to 1115 cm^{-1} (C-O, C-N, and C-C stretching) were also noticed. Spectra show a well-separated strong signal for protein between 1535 and 1570 cm^{-1} arising from amide II and between 1620 and 1690 cm^{-1} due to amide I vibration (Surewicz and Mantsch, 1988; Sarver and Krueger, 1991). The amide I band represents primarily the C=O stretching vibrations of the amide groups coupled to the in-plane N-H bending and C-N stretching modes (Sarver and Krueger, 1991). Belton et al. (1988) also reported that well-separated bands at 1650 and 1540 cm^{-1} arising from protein in confectionery products were obtained using FTIR spectrometer with the aid of a photoacoustic sampling cell. A difference in the spectra at 1100-1300 cm^{-1} arising from C-O stretch vibrations of fat is related to the fat content of cheese samples. The 75% RFCC had the lowest absorption at 1744 cm^{-1} and highest at 1650 cm^{-1} because of its lowest fat and corresponding highest protein contents (Table 3.1). Those of protein-related bands were opposite, highest in the spectrum of 75% RFCC.

Characteristics of spectra of Cheddar cheeses

The peak area of fat A for different fat-level cheeses during aging in the period between 9 to 22 weeks plotted against their fat content measured by proximate analysis is shown in Fig. 3.2. As expected, a change in the peak area during aging is observed for all

Table 3.1--Results of proximate analysis for FFCC and RFCC samples

Content (%)	FFCC	25% RFCC	50% RFCC	75% RFCC
Moisture	42.9 ± 0.3	46.3 ± 0.3	50.0 ± 0.6	52.2 ± 0.6
Protein	20.4 ± 0.5	23.3 ± 0.6	26.1 ± 0.9	30.3 ± 0.7
Fat	32.3 ± 0.3	24.4 ± 0.4	16.9 ± 0.5	8.6 ± 0.4
NaCl	1.3 ± 0.2	1.5 ± 0.4	1.7 ± 0.5	1.7 ± 0.4
Ash	2.3 ± 0.6	2.7 ± 0.6	3.1 ± 0.4	3.4 ± 0.2
pH	5.6 ± 0.2	5.9 ± 0.2	5.9 ± 0.2	6.0 ± 0.3

the cheeses studied, and the mean value increases in proportion to the fat content of cheese. Because the absorption at fat A, obtained by integrating from 1710 to 1820 cm^{-1}, is characteristics of carbonyl groups of various R(CO)OR (1735-1750 cm^{-1}) and R(CO)OH (1760 cm^{-1}) (Silverstein et al., 1991), a decrease in the number of carbonyl groups of R(CO)OR caused by enzymatic hydrolysis is accompanied by a decrease in absorbance (van de Voort et al., 1987); an increase in the number of carbonyl groups of R(CO)OH caused by enzymatic lipolysis and proteolysis led to an increase in absorbance of fat A. For 75% RFCC (8.6% fat) and 50% RFCC (16.9% fat), the peak area of fat A increased during the ripening time between 9 and 22 weeks. This indicates that accumulation of carbonyl groups of various R(CO)OH groups formed due to proteolysis and lipolysis is more significant than the corresponding decrease caused by lipolysis.

In the case of FFCC (32.3% fat) and 25% RFCC (24.4% fat), the peak area of fat A increased within 14 weeks of aging, and then decreased in 22 weeks (Fig. 3.2). The

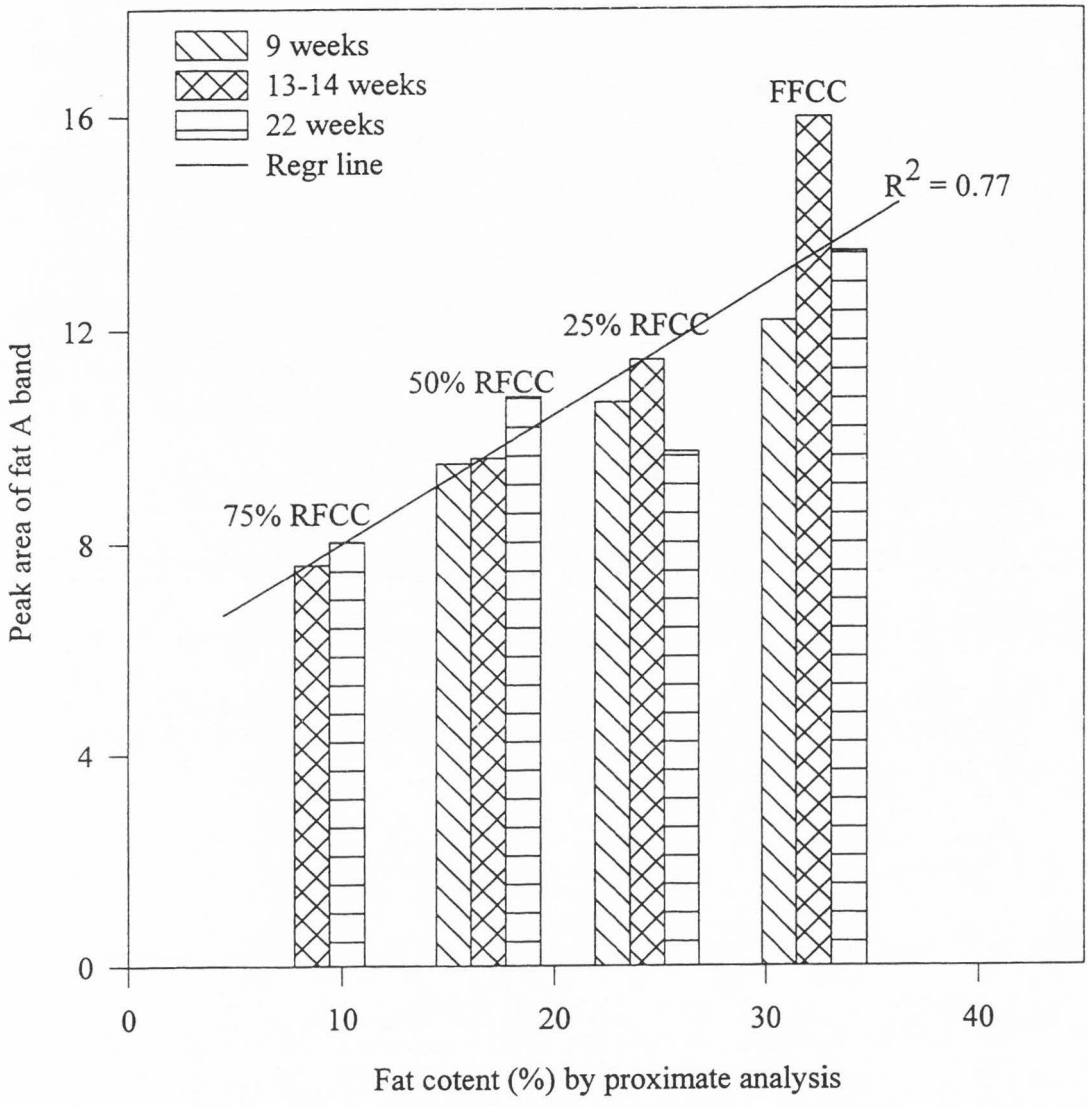

Fig. 3.2--Plot of peak area of fat A band from the spectra of FFCC and 25%, 50%, and 75% RFCCs during aging.

trend observed in FFCC indicates that the decrease of carbonyl groups of various R(CO)OR (detected in the region 1735-1750 cm^{-1}) by lipolysis is more significant than the increase in R(CO)OH (1760 cm^{-1}) caused by both proteolysis and lipolysis after 14 weeks of aging. A regression on the data of peak area at fat A band in the spectra for all cheeses shows that the mean value of peak area of fat A band for each cheese is proportional to the fat content in cheese (R^2 value is 0.77).

Area value of the amide I (Fig. 3.3) and amide II (Fig. 3.4) absorption bands was correlated with protein content of cheese samples determined by proximate analysis (Table 3.1). The peak area of both amide I and II changed during maturing of cheese. The rate and amount of proteolysis occurring in Cheddar cheeses of different fat levels resulted in an increase in the absorbance area of amide I-related bands during aging from 9 to 22 weeks for 50% and 75% RFCC, but increased until 14 weeks and then decreased in 22 weeks for full-fat and 25% reduced-fat Cheddar cheese (Fig. 3.3). Proteolysis occurring in the cheese samples analyzed is affected by the amount of casein content, amount and activity of chymosin entrapped in cheese, strain and amount of starter culture used, existing non-starter culture, salt level, temperature of storage, and moisture content. The presence of these several constituents has resulted in the different type and amount of casein fragments formed during aging, which affected the absorbance of amide I and II related bands. However, a simple regression on the data of peak area at amide I and II bands in the spectra for all the cheese shows that the mean value of peak for amide bands from the spectra for each cheese is linearly to the protein content in the cheese (both R^2 value is 0.86).

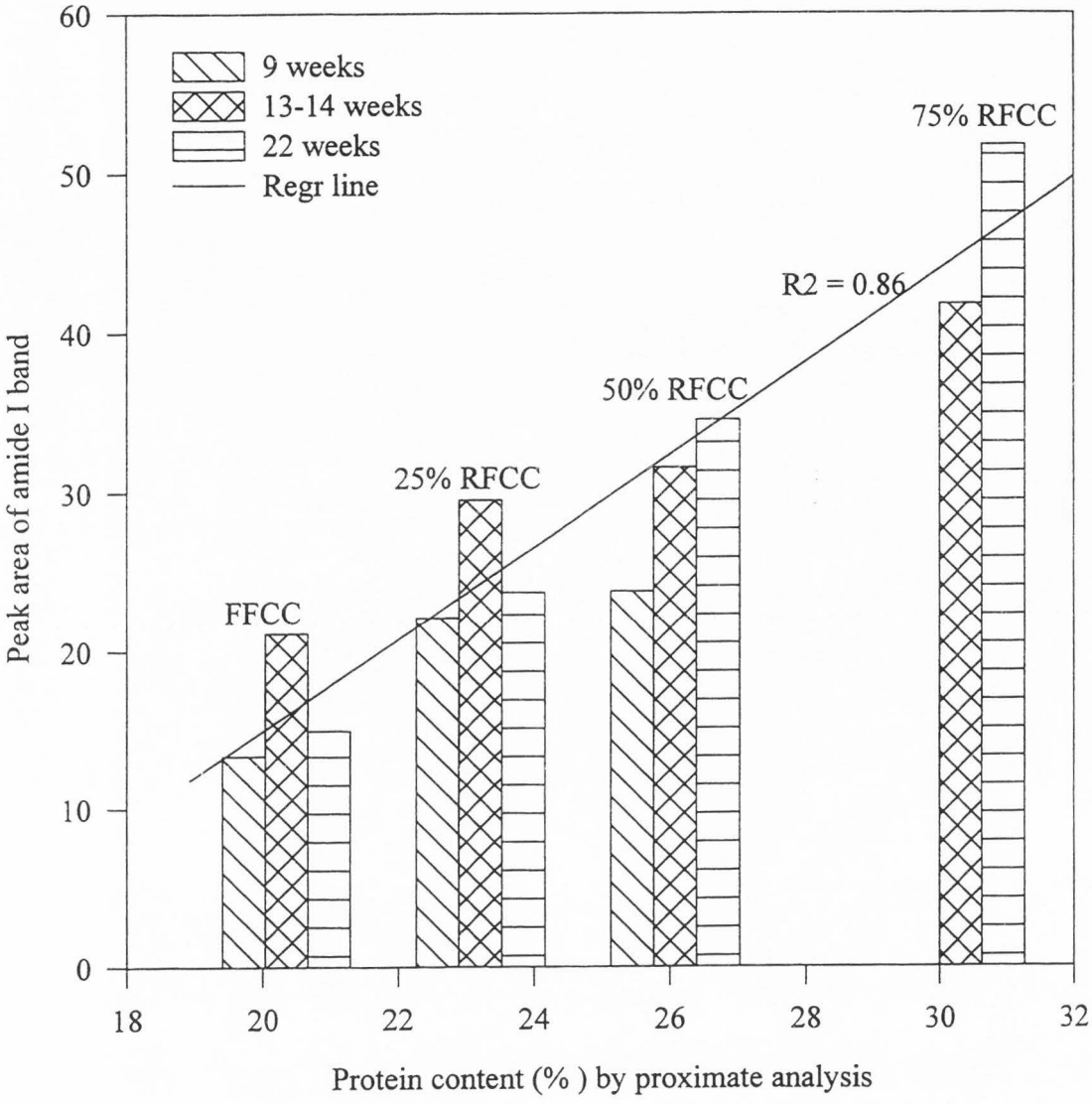

Fig. 3.3--Plot of peak area of the amide I band from the spectra of FFCC and 25%, 50%, and 75% RFCCs during aging.

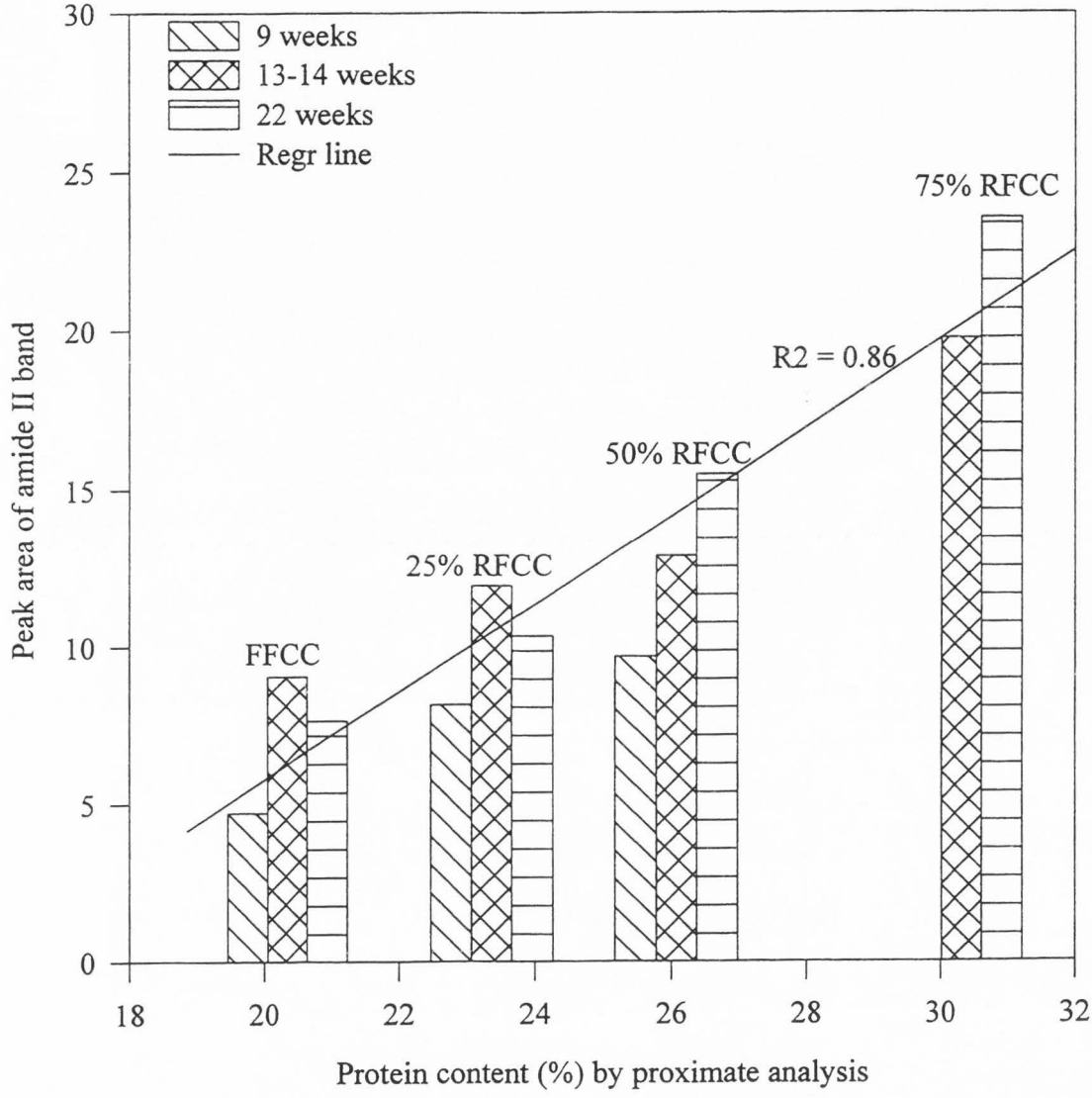

Fig. 3.4--Plot of peak area of the amide II band from the spectra of FFCC and 25%, 50%, and 75% RFCCs during aging.

Table 3.2 lists the results of non-protein nitrogen (NPN) analysis and proteolysis data from capillary electrophoresis (CE) experiments for FFCC (vat 2 only) and RFCC (vat 1 only). An increase in the concentration of NPN content (NPN data from Table 3.2) in reduced-fat and full-fat Cheddar cheeses during aging could be attributed to the accumulation of amino acids and small peptides formed due to the break down of casein during proteolysis. The absorbance at 210 nm (detecting peptide bond) from CE experiments due to α_{s1}-casein (α_{s1}-CN) decreased by 86.0% for FFCC and 84.6% for 75% RFCC during 90 days aging. Comparison of the 90-days-old with the 1-day-old sample indicates that the breakdown of α_{s1}-casein by chymosin present in the coagulant resulted in a 3.2-fold increase in α_{s1}-CN fragment (f24-199) and 4.4-fold increase in α_{s1}-CN fragment (f102-199) during aging. A similar trend was observed by Fox et al. (1994) in their study on proteolysis in cheese during ripening.

As the casein in cheese was broken down into fragments (large or small) and amino acids, a change in the conformation of secondary structure components such as helix, β-sheet, β-turn, and other is possible. The changes in the respective peaks around 1651, 1621 to 1633, 1660 to 1687, and 1644 cm^{-1} corresponding to helix, β-sheet, β-turn/sheet, and other (Surewicz and Mantsch, 1988; Sarver and Krueger, 1991) are noticed in the spectra of the amide I-related band for FFCC (vat 2) (Fig. 3.5) and RFCC (vat 1) (Fig. 3.6) during aging. These bands either decreased or disappeared after aging for 5 months for full-fat Cheddar cheese (Fig. 3.5). In comparison to FFCC, 50% RFCC had a stronger absorption band around 1634, 1650, and 1660 to 1663 cm^{-1} for 5 months than that for 2 months (Fig. 3.6). Further deductions on the secondary structure

Table 3.2.--A comparison of the results of CE and NPN analysis for Cheddar cheese

	Capillary electrophoresis results (Abs $_{210}$)					
	FFCC			75% RFCC		
Time (day)	α_{s1} -CN	α_{s1} -CN (f109-199)	α_{s1} -CN (f24-199)	α_{s1}-CN	α_{s1}-CN (f109-199)	α_{s1} -CN (f24-199)
1	0.017	0.002	0.0022	0.0324	0.005	0.0058
90	0	0.006	0.0098	0.005	0.007	0.013

	NPN analysis results (ml of 0.05 N HCL used/g)	
	FFCC	75%RFCC
65	3.4 ± 0.1	3.8 ± 0.2
90	4.3 ± 0.2	6.1 ± 0.3
155	6.5 ± 0.3	8.0 ± 0.2

components in Cheddar cheese during aging could not be made at this time. Future work in this direction should focus on determining the amount of secondary components and their effect on biochemical interactions during aging. The secondary structure information should also be correlated with other methods, such as the circular dichroism analysis.

CONCLUSION

The spectra of FFCC and all the RFCC (25%, 50%, and 75%) were collected during their aging. The intensity of absorbance for protein- and fat-related bands was compared to the results of proximate analysis. The R^2 was 0.77 for fat and 0.86 for protein-related bands. The NPN content was determined, and the absorbance at 210 nm due to α_{s1}-casein was measured using CE experiments. Comparison of the 90-days-old

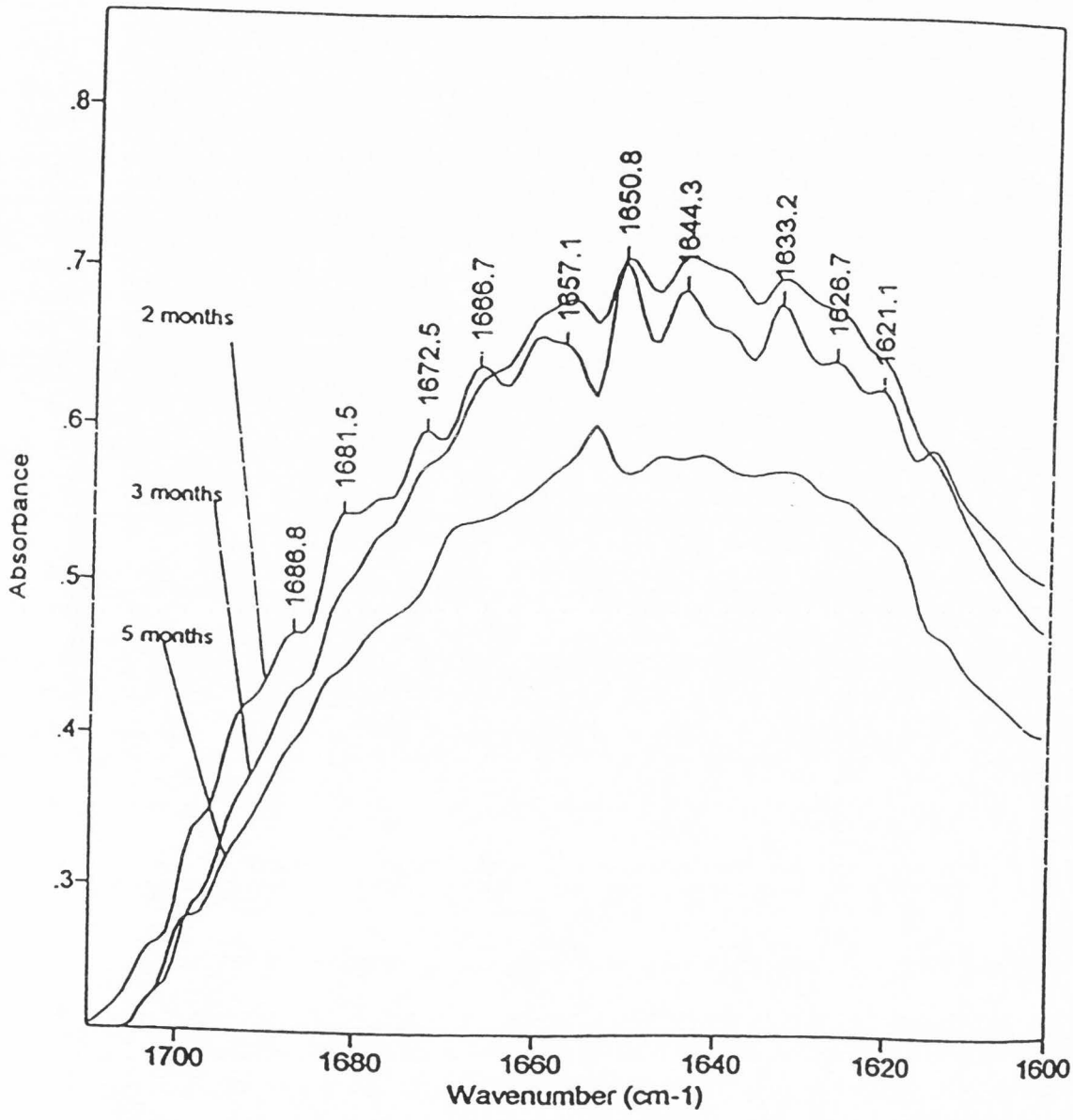

Fig. 3.5--Spectra of FFCCs collected at aging time of 2, 3, and 5 months with the amide I-related bands identified.

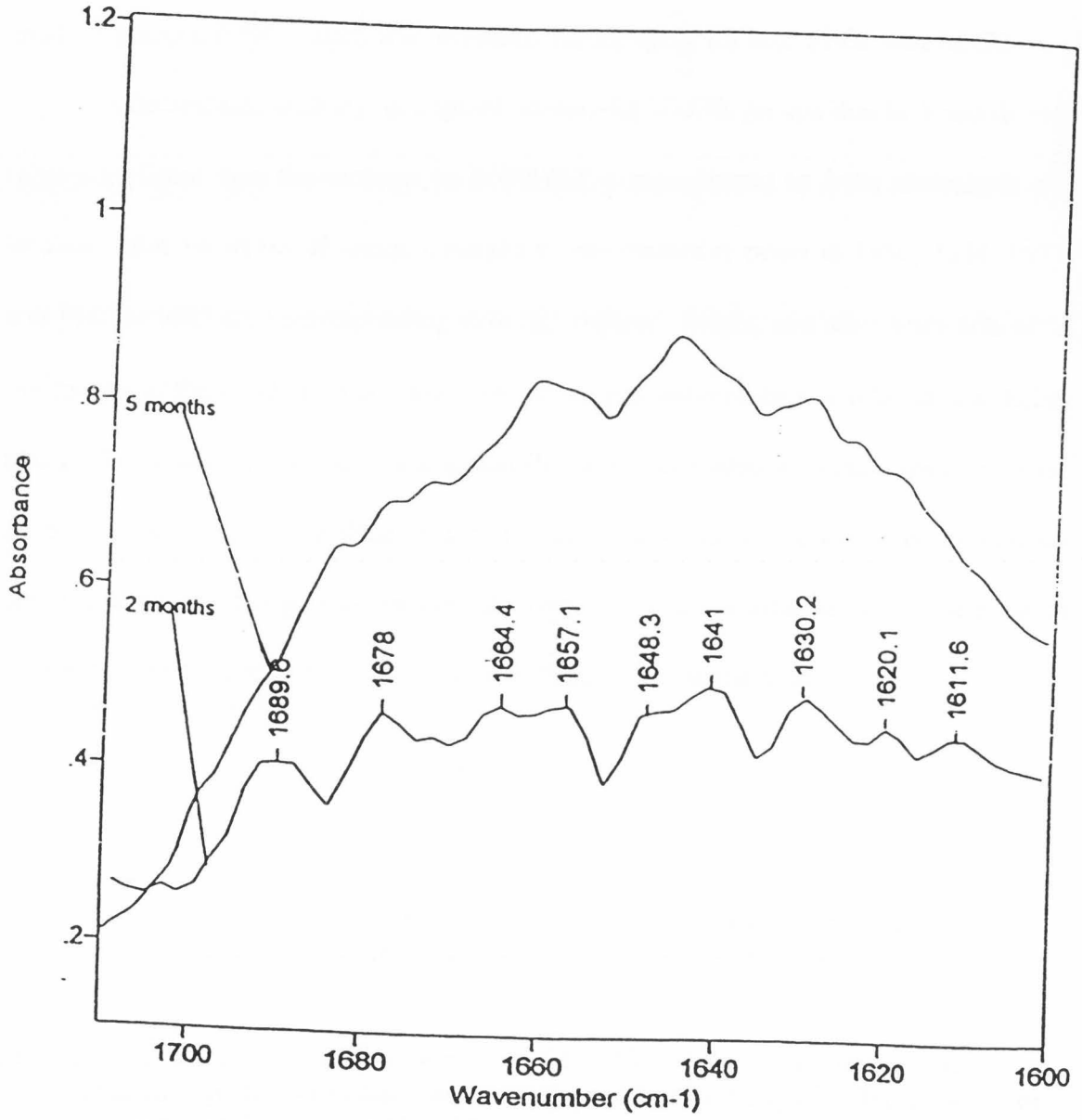

Fig. 3.6--Spectra of 50% RFCCs collected at aging time of 2 and 5 months with the amide I-related bands identified.

with the a 1-day-old sample indicated that the breakdown of α_{s1}-casein by chymosin present in the coagulant resulted in a 3.2-fold increase in α_{s1}-casein f24-199 and a 4.4-fold increase in α_{s1}-casein f102-199 fragments during aging. As a result of the increase of small fragments, NPN content was increased during aging for both FFCC and RFCC.

A decrease in carbonyl groups of various R(CO)OR groups due to lipolysis was more significant than the increase in R(CO)OH groups caused by both proteolysis and lipolysis after 14 weeks of aging. Changes in the respective peaks at 1650, 1634, 1673, and 1660 to 1667 cm^{-1} corresponding to helix, β-sheet, β-turn, and other were noticed in the spectra of the amide I-related band for full-fat and reduced-fat Cheddar cheese during aging. The secondary structure information should be correlated with other methods, such as the circular dichroism analysis. Since the rate and products of lipolysis and proteolysis are important for flavor and texture development, this information could help us to understand the biochemical reactions and to monitor cheese maturation.

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CHAPTER 4

STUDY ON CHARACTERISTICS OF RIPENED CHEDDAR CHEESE AND ITS RIPENING INDEX BY FTIR SPECTROSCOPY

ABSTRACT

Fourier transform infrared spectroscopy coupled with microtome sampling technique was used to analyze characteristics of ripened Cheddar cheese. Absorbance of bands arising from fat- and protein-related functional groups varied during cheese ripening. Change in absorbance of bands at 1744 and 2850-2930 cm^{-1} arising from ester and C-H bond, and 1650 and 1540 cm^{-1} from protein amide I and II, was greater than other functional group-related bands during cheese aging. Bands at 1116 and 1240 cm^{-1} arising from C-O, C-N, C-C stretch, which can be used to estimate the fat and protein contents in cheese, changed slightly during cheese ripening. A correlation coefficient of 0.97 was obtained for absorbance at bands arising from fat ester and C-O bond (fat A and C band) and 0.93 for bands arising from protein amide I and II. Among bands of fat (fat A and C) and protein (amide I and II), the correlation coefficients were greater than 0.80. A ripening index was obtained by correlating cheese ripening time with key functional groups that change during the ripening process. A ripening index model as a function of selected bands at respective chemical groups provided an empirical model for full-fat and reduced-fat Cheddar cheese (FFCC and RFCC) with an R^2 of 0.83 and 0.59, respectively.

INTRODUCTION

Fourier transform infrared (FTIR) spectroscopy is a flexible analytical tool that uses interferometric methods for obtaining infrared spectra and applies suitable mathematical procedures for processing interferograms via fast Fourier transform algorithms to recover the frequency spectrum (Grasselli, 1984). The basic principles of FTIR spectroscopy have been well described by Griffiths and de Haseth (1986). It has been used to analyze food products, such as cheese (McQueen et al., 1995), meat (Dion et al., 1992), fats and oils (van de Voort et al., 1994), butter/margarine (van de Voort et al., 1992a), sweetened condensed milk (van de Voort et al., 1992b), and sugar/juice (Hopkins and Newberry, 1986). It has also been used to determine the secondary structure of proteins (Surewicz and Mantsch, 1988; Sarver and Krueger, 1991). Mendenhall (1991) reported that a Fourier transform infrared spectrometer equipped with an attenuated total internal reflectance cell could be used to determine the milk components, such as fat, protein, and lactose as well as to detect the adulteration of milk with whey powder. The effects of milk fat variation and lipolysis on the infrared spectrum were also studied using this technique.

FTIR spectroscopy has been used to study secondary structure in protein. Boye et al. (1995) demonstrated that changes in the secondary structure of whey proteins resulting from heat treatment were monitored by FTIR spectroscopy. The structural properties of a protein can be characterized by its primary, secondary, tertiary, and quaternary structures (Garland, 1994). The secondary structure describes the orientation of the protein backbone and can be represented by structural elements such as α-helixes, β-sheets,

turns, and nonordered or irregular structures. FTIR spectroscopy is an established method of studying the secondary structure of polypeptides and proteins in both the solution and solid phase (Ismail et al., 1992). The secondary structure of a protein is reflected in the IR spectrum by the absorbance in the amide I region (Garland, 1994). The amide I band absorbs in the 1620-1690 cm^{-1} region and is primarily due to the stretching vibrations of the carbonyl groups. The absorbance bands around 1635 cm^{-1} are associated with the β-structure, whereas the bands closer to 1653 or 1646 cm^{-1} are associated with the helical portions and random portions of the protein, respectively (Byler and Susi, 1986). A variation in absorbance in their key groups was observed during ripening due to the change and rearrangement of the structural pigments. The proteins in Cheddar cheese undergo extensive proteolysis during ripening (O'Keeffe et al., 1976); about 30% of the total protein in Cheddar is soluble at pH 4.6 and 5% of that in 12% TCA, respectively. Proteolysis in cheese during ripening was described in detail by Fox et al. (1994a). During ripening, the combined action of proteinases and peptidases in hydrolysis of casein leads to the formation of products ranging from polypeptides comparable in size to the intact caseins, through intermediate-sized and small peptides, to free amino acids and their degradation products (Fox et al., 1994a). Gel-electrophoretic analysis has shown that α_{s1}-casein is completely degraded to the primary degradation product α_{s1}-I (Fox and Guiney, 1973; Creamer and Richardson, 1974) in mature cheese, but β-casein undergoes very little proteolysis during ripening (Ledford et al., 1966).

It is important to determine the proteolysis which is significant in the development of cheese flavor and texture (Fox et al., 1994a). Methods for assessing proteolysis were

reviewed extensively by Fox et al. (1994b). Most of the methods developed were elaborate, time consuming, and destructive to cheese physical and chemical properties, such as secondary structure of peptides, polypeptides, and proteins. An approach to quantitatively describe the ripening of cheese in terms of the change in biochemical reactions will be a valuable tool for determining the age of cheese using accelerated ripening methods. Quantifying the change in protein secondary structure elements will provide further insight into the chemical changes of protein.

The aim of this work was to study the biochemical characteristics from the spectra of full-fat and reduced-fat Cheddar cheese (FFCC and RFCC) during ripening. Absorbance in the amide I band has been studied to provide an insight into the difference in the change in secondary structure of casein as it undergoes proteolysis in the FFCC and RFCC systems. Change in absorbance at bands corresponding to fat- and protein-related functional groups has been examined. Information on the change in absorbance at specific bands in the FTIR spectra has been correlated with the ripening time and a ripening index is obtained.

MATERIALS & METHODS

Milk and cultures

Skim milk from the Utah State University Dairy Products Laboratory was standardized to 3.6% and 1.8% with cream using Pearsons equation to produce full-fat and reduced-fat Cheddar cheeses. The culture, C.S.S.® bulk set dairy culture (Mesophilic Lactic Acid Producing Cocci) (CT-C, lot No:961131) from Waterford Foods Inc.

(Millville, UT), was used for cheese-making. The culture was grown in low fat milk (2%) at 30°C for about 5 hr before being used.

Cheddar cheese manufacturing procedure

Full-fat Cheddar cheese (FFCC) and reduced-fat Cheddar cheese (50% RFCC denotes a 50 % reduction in fat) were made in the Gary H. Richardson Dairy Products Laboratory at Utah State University (Logan, UT). Both FFCC and RFCC were made in three separate vats and samples from all the vats were used in the analysis. The manufacturing procedures of the FFCC and RFCC were slightly different, and were listed in Tables A1 and A2.

Proximate analysis

The percentage composition of fat, protein, and moisture was determined using the methods outlined in the standard methods for the examination of dairy products (Marshall, 1993). Fat content was determined using the Babcock method (method 15.8d), moisture content by vacuum oven method (method 15.10A), and protein content using the Kjeldahl method (method 15.12A). Samples were tested in triplicate.

FTIR analysis

Small pieces of samples (15 mm height and 15 mm diameter) were cut from the center of a cheese block and frozen for at least 2 hr at -80 °C. The frozen sample was sliced to a thickness of 16 μm using a IM236 microtome (International Equipment Co., Needham Heights, MA). The sliced film was then placed on the surface of a silver chloride crystal in the light path of the Bio-Rad FTS-7 FTIR spectrometer (Bio-Rad,

Digitlab Division, Cambridge, MA) equipped with a dueterated triglycine sulphate (DTGS) detector. Spectra of samples in the region between 4000 and 400 cm^{-1} were obtained with a resolution of 4 cm^{-1} and a scanning frequency of 32 scans/sample. The spectra of cheese samples were collected at different ripening times: every week during the first month, every 2 weeks during months two and three, and every 4 weeks after 3 months. The collected spectroscopic data were processed to obtain the area of peak for selected functional groups using the peak report option in the Bio-Rad Win-IR software either after adjustment of baseline for the peaks assigned to secondary structure or before adjustment for the bands related to fat and protein (Bio-Rad, Cambridge, MA).

Statistical analysis

Statistical regression analysis was used to correlate the absorbance of main bands of fat A and C, protein amide I and II. Simple regression analysis was used to find the statistical significance of the correlation between the ripening time and absorbance change of bands for fat- and protein-related functional groups. An index for ripening was obtained by correlating the protein- and fat-related bands with ripening time using multiple regression analysis option of the statistical analysis system (SAS Institute Inc., Cary, NC).

RESULTS & DISCUSSION

Spectra of aging cheese

The spectra of full-fat Cheddar cheese taken at aging time of 6, 14, and 28 weeks are shown in Fig. 4.1. An increase in absorbance intensity of spectra was observed during

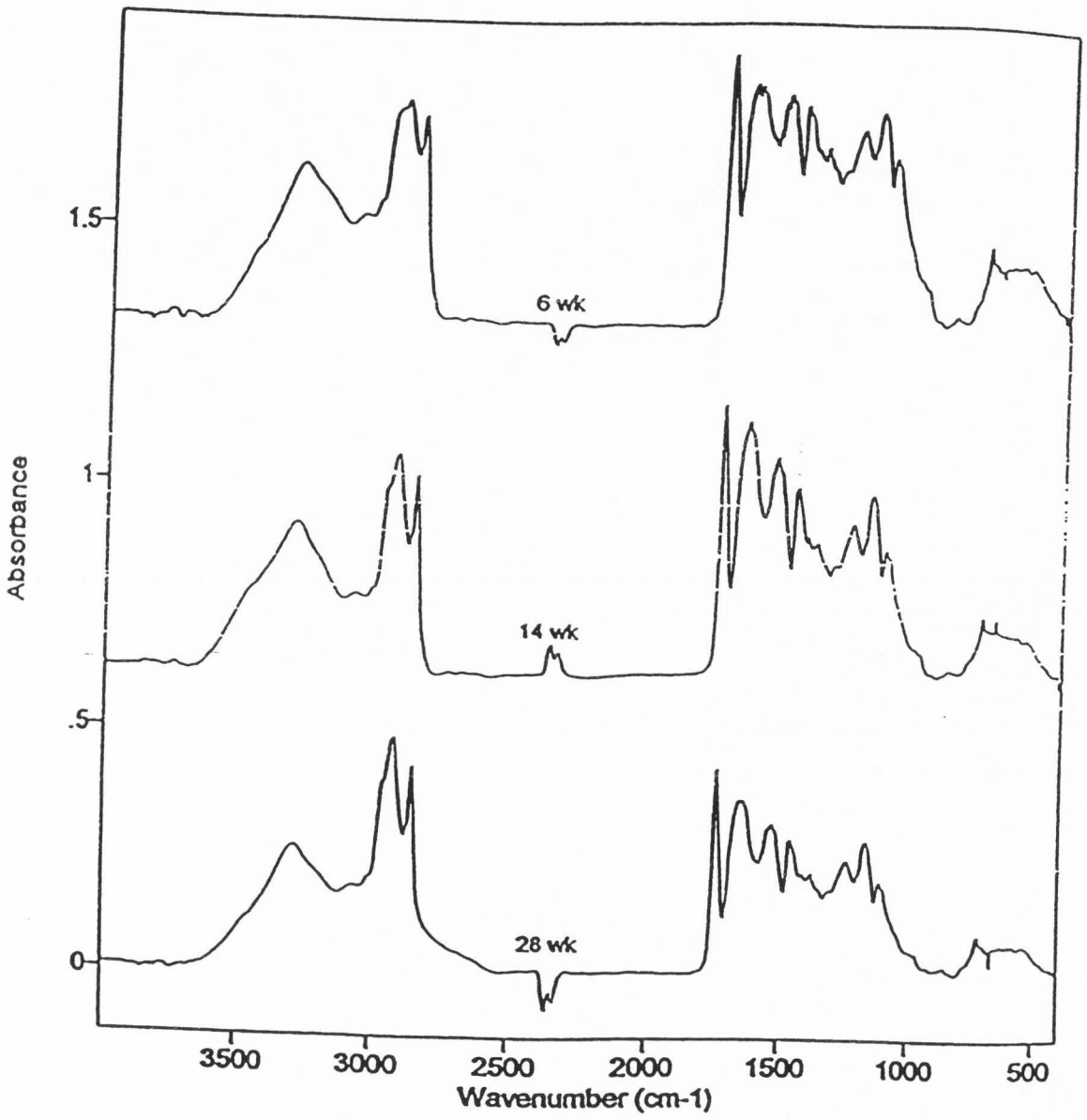

Fig. 4.1--Spectra of aged FFCCs collected after aging for 6 wk, 14 wk, and 28 wk.

cheese maturing. Strong bands at 1744 cm^{-1} from ester carbonyl groups; 2950-2800 cm^{-1} from C-H stretch; 1477-1400 cm^{-1} from $\delta_s CH_2$ (scissoring) of -CH_2CO-, -CH_2-O-, -CH_2-N-, and -CH_2-O-CO- (Silverstein et al., 1991); 1240 and 1195-1129 cm^{-1} from C-C stretch; C-N stretch, and C-O stretch of fat and protein (Belton et al., 1988; Mendenhall, 1991; Silverstein et al., 1991) were well-separated in all spectra. Peaks at around 1650 and 1540 cm^{-1} corresponding to the amide I and amide II of protein, respectively (Susi and Byler, 1988; Surewicz and Mantsch, 1988), were also present. A strong band of water in the range of 3600-3000 cm^{-1} and 1640-1650 cm^{-1} which overlapped with the protein amide I band at 1620-1690 cm^{-1} was noticed. Similar findings were observed in bread (Wilson et al., 1988) and butter (van de Voort et al., 1992a; Safar et al., 1994).

Absorbance characteristics of Cheddar cheese

A change in absorbance peak area at bands around fat and protein groups for all FFCC and RFCC was observed during ripening. The area of peaks corresponding to fat- and protein-related bands for all full-fat and reduced-fat Cheddar cheeses during ripening time up to 28 weeks was obtained using the peak report option of Win-IR software, and the average value of area versus ripening time was plotted in Figs. 4.2 and 4.3. Change was examined in strong bands at 1744 cm^{-1} from ester carbonyl groups (fat A band), 2950-2800 cm^{-1} from C-H stretch (fat B band), and peaks at 1116-1477 cm^{-1} from C-C stretch, C-N stretch, C-O stretch, and $\delta_s CH_2$ (scissoring) in groups of -CH_2CO-, -CH_2-O-, -CH_2-N-, and -CH_2-O-CO- of fat and protein (Belton et al., 1988; Mendenhall, 1991; Silverstein et al., 1991). Peak area of fat A (around 1744 cm^{-1}) and fat B (from 2850 to

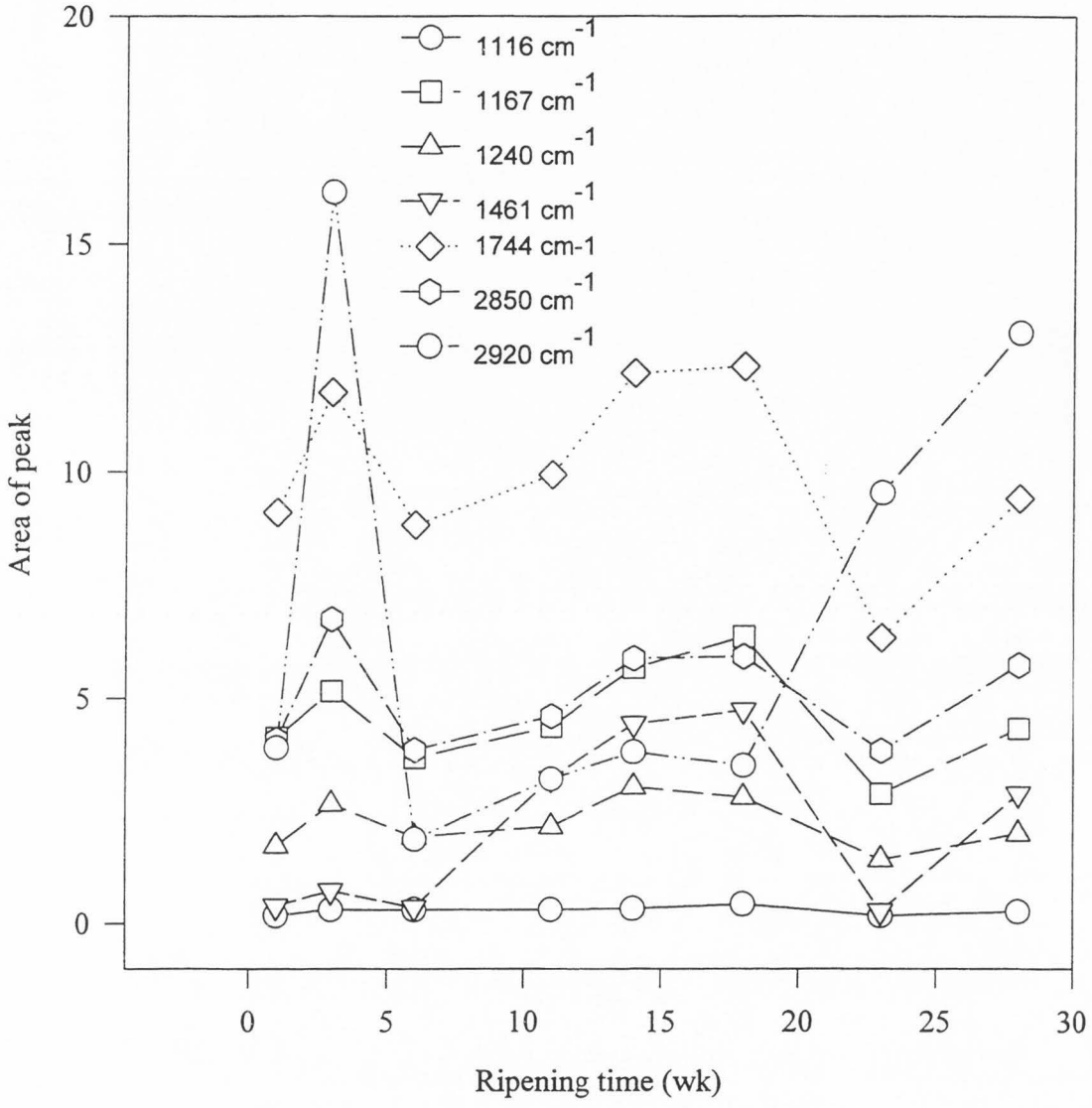

Fig. 4.2--Change in peak area of fat-related bands in FFCC during ripening.

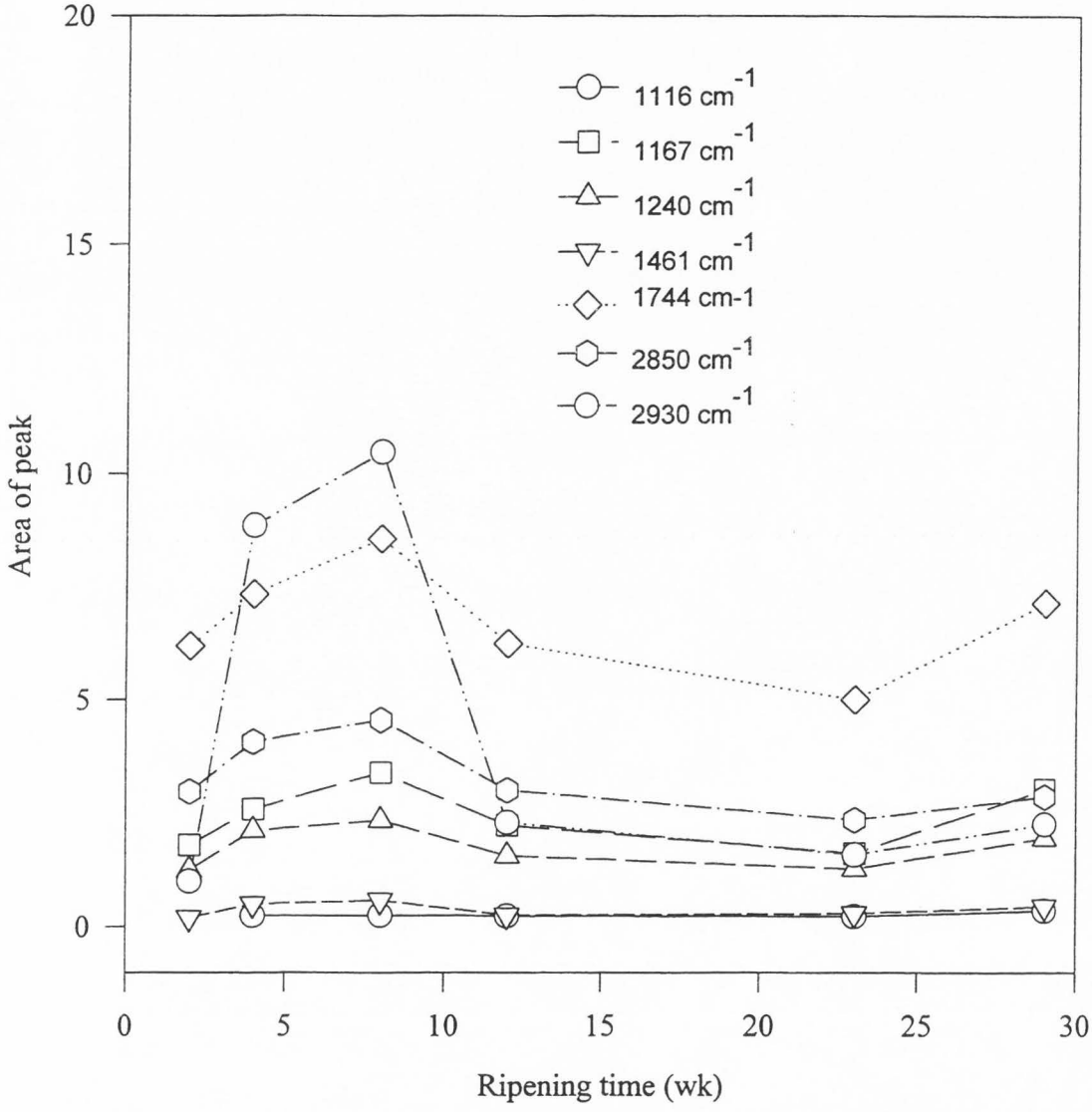

Fig. 4.3--Change in peak area of fat-related bands in RFCC during ripening.

2950 cm^{-1}) varied more drastically with ripening time than other bands because of their sensitivity to fat molecular weight, chain length, saturation, and levels of free fatty acids (Grappin and Jeunet, 1981; Sjaunja, 1982, 1984; van de Voort, 1980). Changes in weight, chain length, saturation, and levels of free fatty acids could be caused from a combination of reactions by catalytic enzymes such as proteinase, peptidase, phosphatase, lipase, decarboxylase, transaminase, redoxase, enzymes that decompose amino acids, and those that undergo respiration and fermentation (Schormüller, 1968).

The absorbance of fat A and B bands for full-fat and reduced-fat Cheddar cheese during ripening is different. This is not surprising because the different microbiological- and enzyme-induced changes occurred in a different composition, pH, moisture, salt level, retained amount of rennet, and starter culture in each cheese (Schormüller, 1968). Not only esters but also carboxylic acids (dimer), aldehydes (dialkyl and aromatic), and ketones (dialkyl and aromatic) contributed to the absorbance around fat A band from 1700 to 1800 cm^{-1} (Silverstein et al., 1991). The increase in absorbance of this band is due to an increase in quantities of these compounds. The amount of these compounds could vary in cheese due to complex biochemical reactions, such as glycolysis, lipolysis, and proteolysis, which occurred during the ripening (Fox et al., 1994a). The enzymes that can catalyze those changes during ripening process are (Scott, 1986) (1) proteases acting on proteins to produce peptides, (2) decarboxylases acting on amino acids to produce amines, (3) decarboxylases acting on keto acids to produce aldehydes, (4) transaminases acting on amino acids to produce keto acids, (5) transaminases acting on keto acids to produce amino acids, (6) deaminases acting on amino acids to produce keto acids, (7)

aminases acting on fatty acids to produce amino acids, and (8) oxidases acting on fatty acids to produce amino acids. Therefore, it is difficult to predict the change of absorbance in fat A and B bands from cheese during ripening.

For the bands from 1110 to 1461 cm^{-1} corresponding to both fat and protein content (Silverstein et al., 1991) in cheese, area of peak at 1461 and 1167 cm^{-1} arising mostly from C-H (scissoring) and C-O (stretch) groups of fat content (Belton et al., 1988; Silverstein et al., 1991) varied to a lesser extent in RFCC compared with FFCC during ripening. The lipolysis due to higher fat content in FFCC than RFCC could be the reason for this difference. The absorbance of bands at 1116 and 1240 cm^{-1} arising from C-C and C-N stretch (Silverstein et al., 1991) changed slightly in both RFCC and FFCC, indicating that the breakdown of these bonds was slow during cheese ripening. Mendenhall (1991) reported that absorption bands from 1110 to 1283 cm^{-1} correlated with fat, protein, and lactose concentrations in milk had a low response to fat variation and lipolysis, and, thus, this range was more suitable to estimate the fat and protein contents in cheese sample.

The average absorbance peak area at amide I (1650 cm^{-1}) and II (1540 cm^{-1}) bands in the spectra for all FFCC and RFCC also varied during ripening (Fig. 4.4). The absorbance trend of these bands during ripening for RFCC is different from that of FFCC due to variation in composition, rate of proteolysis, and rate of lipolysis in each cheese. Change in absorbance of the amide I band is greater than that of the amide II band for both FFCC and RFCC. A probable cause is the effect of varying degrees of protein secondary structure changes (Sarver and Krueger, 1991) and bound water states (van de

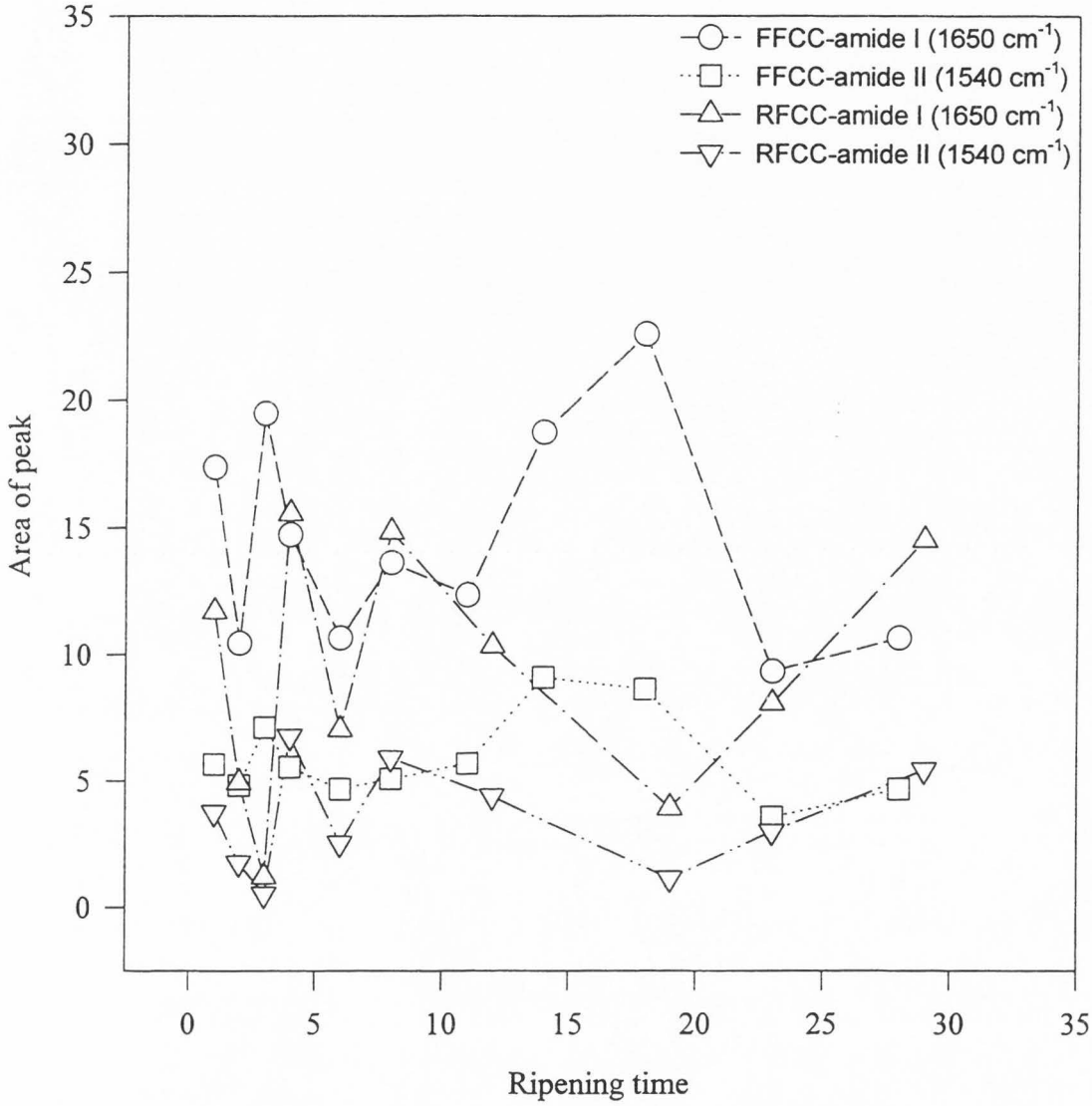

Fig. 4.4--Change in absorbance of peak area at the amide I and the amide II band for FFCC and RFCC during ripening.

Voort et al., 1992a) on the absorbance of these bands during cheese ripening. The formation of free fatty acids from lipolysis of fat (van de Voort, 1980) on the absorbance of the amide II (around 1563 cm^{-1}) band and hydrolysis of protein peptide bonds by proteolysis could be the reason for the variation in the absorbance of amide II for FFCC and RFCC. Data in regard to proteolysis products, bound water and free fatty acids formed from lipolysis in cheese during different ripening stages, are not determined in this work.

The secondary structure of a protein reflected in the IR spectrum by absorbance in the amide I region (1620-1690 cm^{-1}) is primarily due to the stretching vibrations of carbonyl groups (Garland, 1994). The absorbance bands around 1635 cm^{-1} are associated with β-sheet structure, whereas the bands closer to 1653 and 1646 cm^{-1} are respectively associated with the helical and random portions of the protein secondary structure (Susi and Byler, 1988). Area of peaks detected in the amide I region, which was related to secondary structure of casein for FFCC (vat 2) and RFCC (vat 2) during ripening, was obtained using Win-IR software after adjusting the baseline in the spectra (Table 4.1). The peaks at bands 1660-1688 cm^{-1}, corresponding to β-turns/sheets, were observed more often in RFCC than those in FFCC. The total peak area in this range reached the highest level after 11 weeks and decreased or disappeared for both RFCC and FFCC thereafter. Absorbance at 1650-1658 cm^{-1}, corresponding to helix structure, varied with time and is about 5 to 10 times more than usual during 2, 14, and 19 weeks for FFCC. Absorbance in the helix region for RFCC was highest at week 1, decreased till week 4, and could not be detected until week 14. For both FFCC and RFCC, absorbance at 1640 to 1648 cm^{-1}

Table 4.1--Change in absorbance at bands of 1620-1635, 1640-1648, 1650-1658, and 1660-1688 cm^{-1}, corresponding to β-sheet, random, helix, and the turns/sheet protein secondary structure, respectively, for FFCC and RFCC during ripening

Ripening time	β-sheet (1620-1635 cm^{-1})		Random (1640-1648 cm^{-1})		Helix (1650-1658 cm^{-1})		Turns/aheet (1660-1688 cm^{-1})	
(week)	RFCC	FFCC	RFCC	FFCC	RFCC	FFCC	RFCC	FFCC
1	-	-	-	0.29	4.6	2.39	-	-
2	0.1	-	0.13	-	0.48	10.8	0.1	-
4	0.59	0.61	1.03	0.78	0.21	0.24	1.17	0.8
6	0.22	-	0.1	0.41	-	-	0.19	2.05
8	-	-	0.19	0.48	-	1.78	0.33	-
11	-	-	-	-	-	-	10.7	12.5
14	-	-	0.3	-	-	18.1	0.9	-
19	0.1	-	0.1	-	0.41	21.9	0.1	-
23	-	-	0.25	0.51	1.54	1.18	-	-
29	-	-	0.24	-	2.75	2.23	-	-

- represents value not detected.

denoting random segment and at 1611 to 1638 denoting β-sheet (Surewicz and Mantsch, 1988) was observed in lower levels compared to that of helix and turns/sheet but changed randomly during ripening for both cheeses.

The change in secondary structure is due to the breakdown of casein and formation of small polypeptides and fragments from proteolysis during cheese aging. The extent of proteolysis varied, depending upon cheese-making procedure, starter culture, composition of milk and cheese, rennet, salt level, pH, and storage condition (Fox et al., 1994a). These variations, coupled with lipolysis and amount of bound water in different cheese blocks, suggest a greater change of absorbance in the amide I band than was expected. Therefore, the amide I band is not suitable for estimation of protein contents in cheese.

Correlation of absorbance between fat and protein related bands during ripening

The correlation in absorbance among fat-related bands at 1744 cm^{-1} (ester bond-fat A) and 1167 cm^{-1} (C-O stretch-fat C), and protein-related bands at 1650 cm^{-1} (amide I) and 1540 cm^{-1} (amide II) was analyzed based on the spectra data of all FFCC and RFCC. Table 4.2 shows the correlation coefficients for different constituents corresponding to their absorbance wavelengths. A coefficient of 0.97 among fat-related bands and 0.93 among protein-related bands was obtained. The high correlation coefficient between the fat A and the fat C band is due to the effect of lipolysis of fat during ripening. A correlation coefficient of 0.93 between protein amide I and amide II indicates that one of these protein groups is highly correlated to the other due to the effect of proteolysis on

Table 4.2.--Degree of correlation among fat and protein bands for Cheddar cheese during aging

Bands	Fat A (1744 cm^{-1})	Fat C (1167 cm^{-1})	Amide I (1650 cm^{-1})	Amide II (1540 cm^{-1})
Fat A	1.00	0.97	0.87	0.91
Fat C	0.97	1.00	0.86	0.88
Amide I	0.87	0.86	1.00	0.93
Amide II	0.91	0.88	0.93	1.00

their absorbance during ripening. The correlation coefficient between fat- and protein-related bands is 0.87 for fat A and amide I band and 0.91 for fat A with amide II band, and 0.86 and 0.88 for fat C with amide I and amide II bands, respectively. These data provide valuable insight with respect to the intensity of interaction between fat- and protein-related bands.

Correlation of aging time and absorbance of functional groups

The correlation of ripening time with absorbance of each peak area arising from different function groups for FFCC and RFCC was analyzed with SAS and the results are tabulated (Tables 4.3 and 4.4). The band at 1461 cm^{-1} arising from $\delta_s CH_2$ (scissoring) of $-CH_2CO-$, $-CH_2O-$, $-CH_2N-$, and $-CH_2-O-CO-$ (Silverstein et al., 1991) is highly correlated to aging time for FFCC. But aging time is more correlated to the band at 1116 cm^{-1} arising from C-O, C-N, and C-C stretches for RFCC. High correlation between ripening time and absorbance at 1116 cm^{-1} for RFCC and 1461 cm^{-1} for FFCC (its R^2 is also the highest at 0.16 and 0.56, respectively) indicates that a change in these functional

Table 4.3--Correlation coefficients for ripening time versus absorbance of peak area for key functional groups in FFCC during aging

Wavelength of peak (cm^{-1})	Related functional groups	Correlation coefficient
1116	C-N & C-C stretch	-0.183
1167	C-O stretch	0.521
1240	C-C & C-N stretch	0.448
1461	$-CH_2X$, X=CO, O, N, OCO	0.746
1744	R(CO)X, X= O^-, OR, H, R	0.549
2850	$R(CH_2)R$	0.06
2930	CH_3R	0.301
1530-1540	amide I (peptide bond)	0.144
1640-1650	random portion	0.022
1653-1658	helical portion	-0.008

Time (wk) = 3.22 - 0.30 * 1116cm^{-1} - 5.40 * 1167cm^{-1} + 7.54 * 1240cm^{-1}
+ 7.21 * 1461cm^{-1} - 0.01 * 1744cm^{-1} + 0.34 * 2930cm^{-1}
- 2.04 * 1530-1540 cm^{-1}

R^2: 0.83

Root MSE: 4.88

Table 4.4--Correlation coefficients for ripening time versus absorbance of peak area for key functional groups in RFCC during aging

Wavelength of peak (cm^{-1})	Related functional groups	Correlation coefficient
1116	C-N & C-C stretch	-0.402
1167	C-O stretch	0.06
1240	C-C & C-N stretch	0.224
1461	$-CH_2X$, X=CO, O, N, OCO	-0.133
1744	R(CO)X, X= O^-, OR, H, R	0.257
2850	$R(CH_2)R$	-0.329
2930	CH_3R	0.031
1530-1540	amide I (peptide bond)	-0.092
1640-1650	random portion	0.047
1653-1658	helical portion	-0.138

$$\text{Time (wk)} = 18.68 - 6.98 * 1116cm^{-1} - 10.96 * 1167cm^{-1} + 40.73 * 1240cm^{-1} + 0.78 * 1461cm^{-1} - 6.38 * 1744cm^{-1} - 0.60 * 2930cm^{-1} - 4.10 * 1530\text{-}1540cm^{-1} + 1.31 * 1653\text{-}1658\ cm^{-1}$$

R^2: 0.59

Root MSE: 8.07

groups with time has a greater effect on ripening. For both FFCC and RFCC, a very low correlation is obtained for the bands in the range of amide II and amide I. This shows that absorbance change of these bands is not linear with respect to ripening time since their absorbance is affected by the combined action of the proteinases and peptidases during the hydrolysis of casein (Fox et al., 1994a). Multiple regression analysis was used to estimate the ripening index models based on the bands that showed a high correlation. An R^2 of 0.83 for FFCC and 0.59 for RFCC was obtained for the best fit linear regression models. The low value of R^2 for RFCC could be due to the reduction in fat content and increase in protein content, which affects the correlation of ripening time and absorbance in band at 1461 cm^{-1} from C-H scissoring of $-CH_2O-$, $-CH_2CO-$, $-CH_2N-$, and $-CH_2-O-CO-$ groups. The proposed analysis and methodology provide valuable insight with respect to absorbance and cheese ripening through simple regression analysis of spectral data. This methodology can be adopted to study the ripening of new cheese varieties using accelerated ripening methods.

CONCLUSION

Variation in absorbance of bands at 1116, 1167, 1240, 1461, 1744, 2850, 2930, 1650, and 1540 cm^{-1} during cheese aging was observed. Absorbance of fat-related bands at 1167, 1744, 2850, and 2930 cm^{-1}, and protein-related bands in the range of 1500-1580 and 1600-1690 cm^{-1} changed greatly during ripening predominantly due to proteolysis and lipolysis. Relatively, absorbance at bands from 1100-1240 cm^{-1} changed slightly, which could be suitable for quantitative analysis of fat and protein contents in cheese.

Variation in the bands at 1620-1635, 1640-1648, 1650-1558, and 1660-1688 cm^{-1} assigned to β-sheet, random, helix, and the turns/sheet portion of secondary structure of protein, respectively, was observed for RFCC and FFCC during ripening. The predominance of the bands at 1650-1658 and 1660-1688 cm^{-1} due to the helical and turns/sheet portion of secondary structure indicated that these structures were more stable than other secondary structures found in protein (Damodaran, 1996). Absorbance change of these bands could be due to the formation of various small polypeptides produced during the breakdown of casein by rennet and proteinases during ripening of 1 to 29 weeks. The analysis results indicated that different polypeptides, small peptides and segments, and free amino acids were formed during the breakdown of casein by proteolysis in each cheese during the cheese-making and ripening stages.

Correlation coefficients of greater than 0.93 were obtained among fat- or protein-related bands. A correlation of greater than 0.85 was obtained between fat- and protein-related bands, indicating that there was a strong interaction between protein and fat. Ripening index was obtained using simple regression analysis based on the bands that showed a high correlation with ripening time. A maximum R^2 of 0.83 for FFCC and 0.59 for RFCC was obtained for the respective ripening index regression models. This methodology could be adopted to study the ripening of new cheese varieties using accelerated ripening methods. The models could be improved if more samples could be obtained and data collected beyond 28 weeks of ripening.

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CHAPTER 5

TEXTURE DEVELOPMENT AND ITS RELEVANCE TO BIOCHEMICAL CHANGES IN LOW-FAT CHEDDAR CHEESE DURING RIPENING

ABSTRACT

Texture development and change in biochemical groups in full-fat and reduced-fat Cheddar cheese during ripening were studied. Texture parameters such as hardness, gumminess, chewiness, springiness, adhesiveness, and cohesiveness were monitored. Change in main chemical groups was determined from the Fourier transform infrared spectra. Hardness, gumminess, and chewiness had the same trend during cheese aging, which decreased in the early stages then increased with aging time. Cohesiveness and springiness changed slightly with the age of cheese. Adhesiveness decreased with aging time for both full-fat and reduced-fat Cheddar cheese. A slight increase in cohesiveness and adhesiveness was observed as fat content of cheese decreased from 31% to 21%. The difference in springiness between FFCC (31.3% fat content) and RFCC (21.4% fat content) was not significant using a texture profile test. Multiple regression analysis was used to determine functional relationship between hardness, adhesiveness, and springiness with respect to changes in main chemical groups from FTIR spectra. An R^2 value of 0.67, 0.54, and 0.75 was obtained for hardness, adhesiveness, and springiness for full-fat Cheddar cheese; values of 0.51, 0.59, and 0.54 were obtained for reduced-fat Cheddar cheese, respectively. Correlation of cheese texture with biochemical changes has

provided valuable insight and understanding of texture development. This could be used as a tool for quantifying texture in terms of biochemical changes.

INTRODUCTION

Texture is an important characteristic of Cheddar cheese in deciding consumer acceptability and quality (McEwan et al., 1989). Much research has been published on cheese texture (Chen et al., 1979). It is known that reduction in fat content usually resulted in excessively firm and elastic (often described as "rubbery"), or hard, dry, and possibly grainy cheese (Czulak and Spieler, 1973). This is because there is more structural matrix per unit cross-sectional area in reduced-fat cheeses (Emmons et al., 1980), which in turn allows the syneresis process to accelerate further during cheese manufacture due to the relative deficiency of fat globules.

The structural matrix of cheese is a cross-linked casein-calcium phosphate network in which fat globules are physically entrapped (Lawrence et al., 1983). Scanning electron micrographs indicted that the nature of the protein matrix, which was affected by fat content of the cheese, influenced texture attributes (Bryant et al., 1995). Hardness and springiness increased with decreasing fat content. The matrix is elastic when the casein is largely intact, but its elasticity is lost as proteolysis proceeded during cheese maturation. The entrapped fat globules serve to limit deformation of the elastic cheese matrix, and their size distribution is a function of the breed of cow and shear history of the milk, which in turn determines the uniformity and degree of cross-linking of the casein matrix (Jameson, 1990).

Many investigators have studied the effects of cheese composition and protein breakdown on instrumental measurements of the rheology of various types of cheese (Chen et al., 1979; Fedrick et al., 1986). Lawrence et al. (1983) pointed out that differences in texture at any particular stage of ripening depend on the differences in basic structure and the extent to which the basic structure has been modified. Johnston et al. (1994) studied the electrophoretic pattern and textural assessment of aging Cheddar cheese made with various levels of calf rennet or microbial coagulant. Results indicated that cheese texture primarily depends upon cheese pH, chemical composition, and subsequent changes in texture determined by α_{s1}-casein breakdown. Strong correlation was obtained (R^2 = +0.800, -0.914 and -0.651) between α_{s1}-casein breakdown and changes in curdiness, stickiness, and smoothness with time, but a basis for increase in smoothness and decrease in curdiness with increasing set-to-cut time was not provided. However, increasing the set-to-cut time from 30 to 50 min significantly increased the cheese moisture content, pH, and calcium level and, consequently, cheese texture. Subsequent, early changes in texture were determined by α_{s1}-casein breakdown.

Johnson and Chen (1995) pointed out that firmness of cheese is not controlled solely by its composition. The ionic interactions between protein strands influenced by the pH and bound calcium play a major role in the firmness of cheese. However, the most important contributing factor to the firmness of cheese is the level of proteolysis that occurs during ripening. The products of proteolysis, i.e., amino acids and peptides and, especially, compounds derived from them, are known to contribute to texture and flavor of cheese (both desirable and undesirable).

Casein, particularly the α_s-moiety, is hydrolyzed first, whereas β- and ρ-κ-casein are not greatly proteolysed in most bacteria-ripened cheeses (Nauth and Ruffie, 1995). Also, texture development during aging occurs in two phases (Lawrence and Gilles, 1987). *Phase one* constitutes the first 7-14 days when the rubbery texture of young cheese is rapidly converted to a smoother, more homogenized product as a result of breakdown of α_{s1}-casein. *Phase two* involves a more gradual change in texture over the months as a result of continuing breakdown of α_{s1}-casein and other caseins.

Work has been done in the past to relate texture and rheological properties to composition, pH, salt level, and rennet used (Lawrence and Gilles, 1987). However, very limited work has been done to study and compare texture development in full-fat and reduced-fat cheese and its relation to biochemical changes during aging. The work presented has specifically (1) addressed the difference in texture development between full-fat and reduced-fat Cheddar cheese (FFCC and RFCC) by monitoring the texture during different stages of ripening; (2) monitored changes in key chemical groups observed at respective wavelengths from FTIR spectra; and (3) quantified texture in terms of change in key chemical groups.

MATERIALS & METHODS

Milk and cultures

Skim milk from the Utah State University Dairy Products Laboratory was standardized to 3.6% and 1.8% with cream using Pearsons equation to produce full-fat and reduced-fat Cheddar cheeses. The culture, C.S.S.® Bulk Set Dairy cultures

(Mesophilic Lactic Acid Producing Cocci) (CT-C, lot No:961131) from Waterford Foods Inc. (Millville, UT), was used for cheese. The culture was grown in low fat milk (2%) at 30°C for about 5 hr before its use.

Cheddar cheese manufacturing procedure

Full-fat Cheddar cheese (FFCC) and reduced-fat Cheddar cheese (50% RFCC) (fat reduction: 50%) were made in the Gary H. Richardson Dairy Products Laboratory at Utah State University (Logan, UT). Both FFCC and RFCC were made in three separate vats and the samples from all the vats were used in the analysis. The manufacturing procedures for the FFCC and RFCC are listed in Tables A1 and A2.

Proximate analysis

The percentage compositions of fat, protein, and moisture were determined using the methods outlined in the standard methods for the examination of dairy products (Marshall, 1993). Fat content was determined using the Babcock method (method 15.8d), moisture content by vacuum oven method (method 15.10A), and protein content was determined using the Kjeldahl method (method 15.12A). Samples were tested in triplicate.

FTIR analysis

Small samples (15 mm in height and 15 mm in diameter) were cut from the center of a cheese block and frozen for at least 2 hr at -80 °C. The frozen sample was sliced to thin samples (16 μm thick) using a IM236 microtome (International Equipment Co., Needham Heights, MA). The sliced film was then placed on the surface of a silver

chloride crystal in the light path of the Bio-Rad FTS-7 FTIR spectrometer (Bio-Rad, Digitlab Division, Cambridge, MA) equipped with a deuterated triglycine sulphate (DTGS) detector. The samples were allowed to equilibrate for at least 10 min, and spectra of samples in the region between 4000 and 400 cm^{-1} were obtained with a resolution of 4 cm^{-1} and a scanning frequency of 32 scans/sample. The spectra of cheese samples were collected at different aging time: every week during the first month, every 2 weeks between months two to three, and every 4 weeks after 3 months. The collected spectroscopy data were processed, and the area of peak for selected functional groups is obtained using the peak report option in the Bio-Rad Win-IR software (Bio-Rad, Cambridge, MA).

Texture profile analysis (TPA)

A cylindrical probe of 1-inch diameter was used for all the tests. Cylindrical samples of 15-mm diameter, and 15-mm height (Jack et al., 1993), were chosen for all texture tests. The primary consideration in sampling is representation, homogeneity, and suitability to accommodate the entire diameter of the probe. These samples were large enough to represent the whole block and at the same time small enough to avoid the inclusion of structural irregularities (Prentice, 1992). All the samples were cut at 4°C using a cork borer, to prevent barreling of the cylinders. The samples were obtained from the middle of the whole cheese block rather than from the surface. The firmness of cheese at the surface of the block is expected to be relatively greater than that in the middle, due to the effect of surface drying (Prentice, 1992). Texture and spectra data of 50% reduced-fat and full-fat Cheddar cheese were obtained simultaneously for different ripening times.

A Steven Farnells QTS-25 texture analyzer was used for measuring the texture properties of cheese using the TPA option. The instrument can be operated with the help of an interactive Windows-based program which calculates and provides absolute values for product texture parameters (hardness, cohesiveness, adhesiveness, gumminess, chewiness, and springiness). Speed of the probe is 10 mm/min, and the deformation is set at 20%. The samples were allowed to equilibrate in room temperature for 1 hr in a closed container (Marshall, 1990) before the tests. All tests were done at room temperature in replicates of five.

Statistical analysis

Correlation of texture development data and spectral absorbance changes of main bands in the spectra of Cheddar cheese were obtained using a statistical analysis system. Multiple regression analysis was used to quantify cheese hardness, springiness, and adhesiveness with the highly correlated bands using the statistical analysis system (SAS Inc., Cary, NC).

RESULTS & DISCUSSION

TPA analysis

Hardness, the force necessary to attain a given deformation, is one of the important factors in determining cheese texture (Bryant et al., 1995). Reduction of fat content in cheese increased the hardness of cheese (Fig. 5.1). However, the trend of hardness for FFCC and RFCC during ripening had a similar pattern. Hardness decreased within 4 to 6 weeks, and increased after that. The decrease in hardness during the early

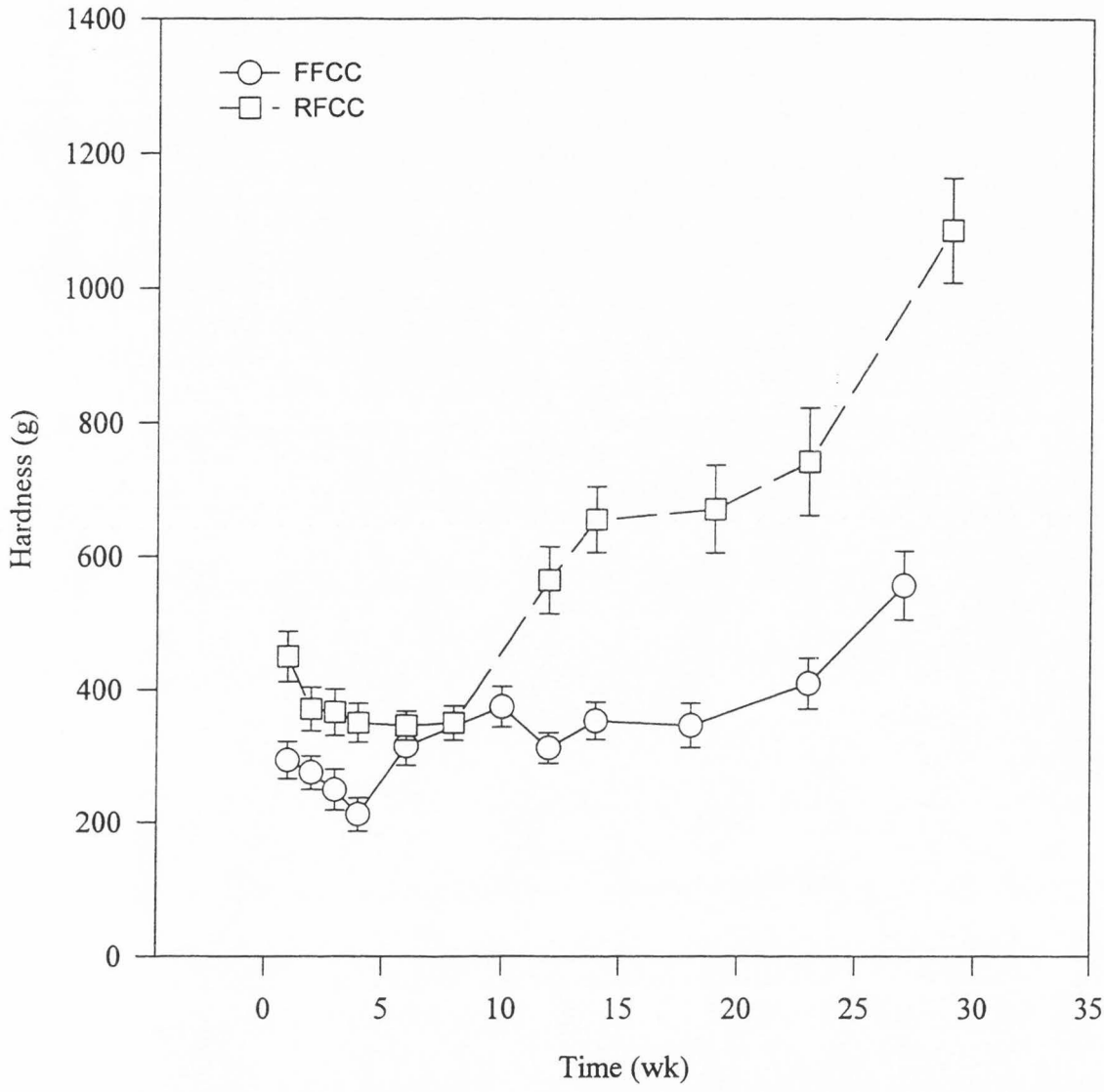

Fig. 5.1--Change in hardness of FFCC and RFCC during ripening.

stages of ripening is due to the initial rubbery texture of young cheese curd, which rapidly transforms into a smoother, and a more homogeneous product due to proteolysis of the casein network. This casein network was greatly weakened when only a single bond in about 20% of the α_{s1}-casein was hydrolyzed by the coagulant to give the peptide α_{s1}-I (Creamer and Olson, 1982). The increase in hardness value after 4-6 weeks is mainly due to the status of water during proteolysis (Lawrence and Gilles, 1987). As each peptide bond is cleaved, two new ionic groups are generated, and each of these competes with the available water in the system. Thus, the water previously available for solvation of the protein chains will become tied to the new ionic groups. Relatively low moisture cheese, such as Cheddar, tends, therefore, to become increasingly harder with age and more resistant to small deformations (Creamer and Olson, 1982). The composition of FFCC and RFCC, given in Table 5.1, reports a higher moisture content for RFCC. Change in hardness indicates that this minor increase in moisture content does not sufficiently compensate for the higher protein content in cheese. The increase in hardness of RFCC is more pronounced with age as more ionic groups compete with the available water. Lawrence and Gilles (1987) in a similar study concluded that the lower the ratio of moisture to casein, the firmer will be the casein matrix of the cheese. The rate of proteolysis is controlled largely by the proportion of residual rennet and plasmin in the cheese, salt-to-moisture ratio, and storage temperature. The rise in pH that occurs during ripening is another factor that might affect the overall hardness.

Cohesiveness is the ratio of the positive force area during the second compression to that during the first compression (Figure B1). The mean values for both FFCC and

Table 5.1--Composition of FFCCs and RFCCs

Compositions	Sample and percentage of compositions	
	FFCC	RFCC
Fat	31.3 ± 0.2	21.4 ± 0.2
Protein	17.2 ± 0.3	21.8 ± 0.3
Moisture	38.5 ± 0.8	42.4 ± 0.4
Ash	2.7 ± 0.4	3.4 ± 0.2

RFCC (Fig. 5.2) indicate a slight increase in cohesiveness as fat content of cheeses decreased from 31.3% to 21.4%. Bryant et al. (1995) reported that as fat was removed, the cheese became more springy. In this state, the resistance to deformation is higher and, hence, does not rupture easily. Therefore, RFCC is more cohesive than FFCC. During cheese ripening, cohesiveness for both full-fat and reduced-fat cheese changed slightly (Fig. 5.2). The increase in cohesiveness with a decrease in fat can also be attributed to an increase in the area of protein matrix and an increased presence of moisture in the cheese system.

Gumminess, the product of hardness and cohesiveness, increased in proportion to hardness and cohesiveness, as fat content decreased (Fig. 5.3). Change in gumminess during ripening is similar to the change in hardness for both full-fat and reduced-fat cheese for similar reasons deduced for hardness.

Adhesiveness, which is the work necessary to overcome attractive forces between the surface of cheese and surface of contracting material, decreased slightly with decreasing fat content from 31 to 21% (Fig. 5.4). A suggested reason is that the decrease in fat contents in RFCC resulted in an increase in protein content during manufacture.

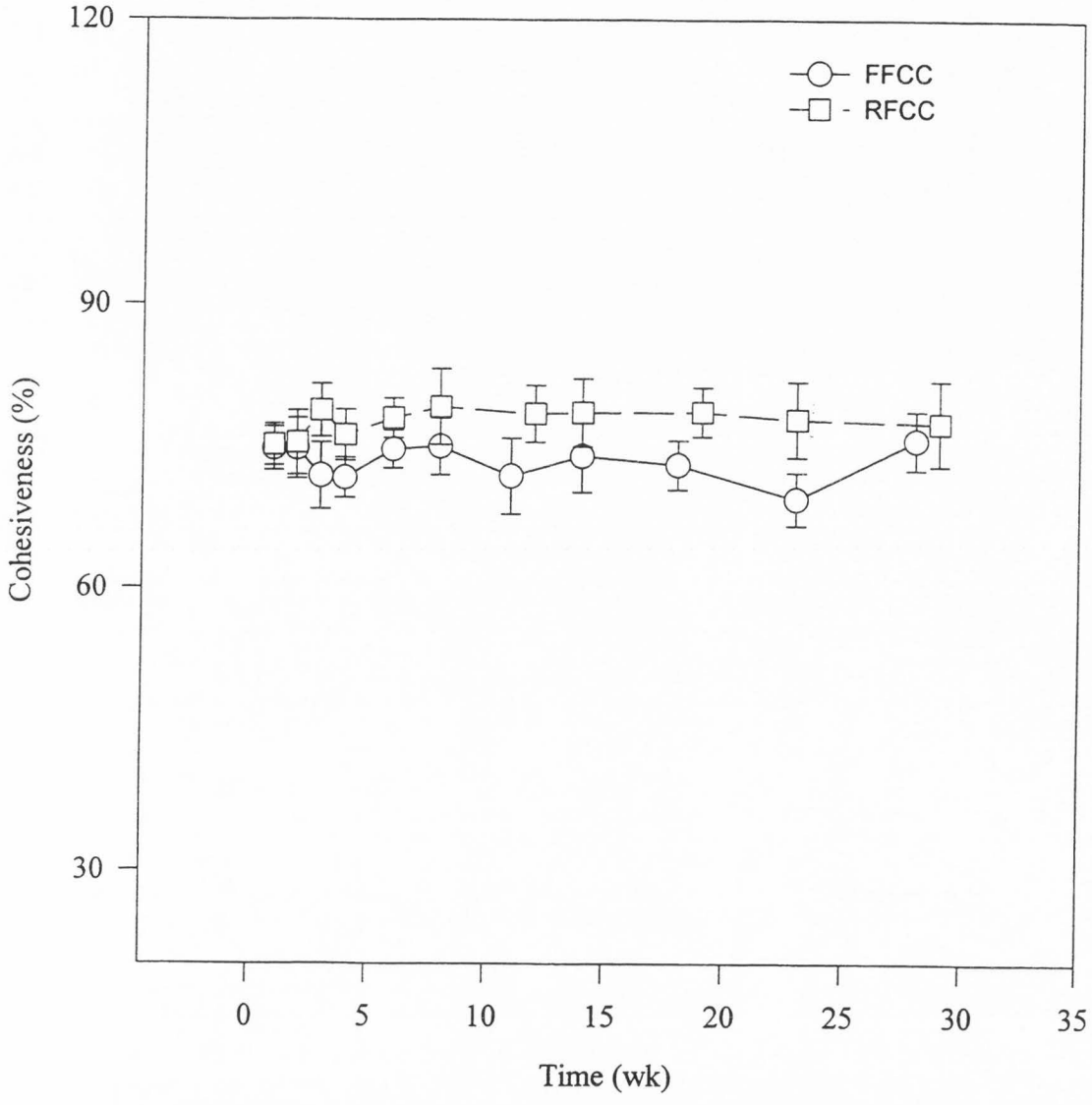

Fig. 5.2--Change in cohesiveness of FFCC and RFCC during ripening.

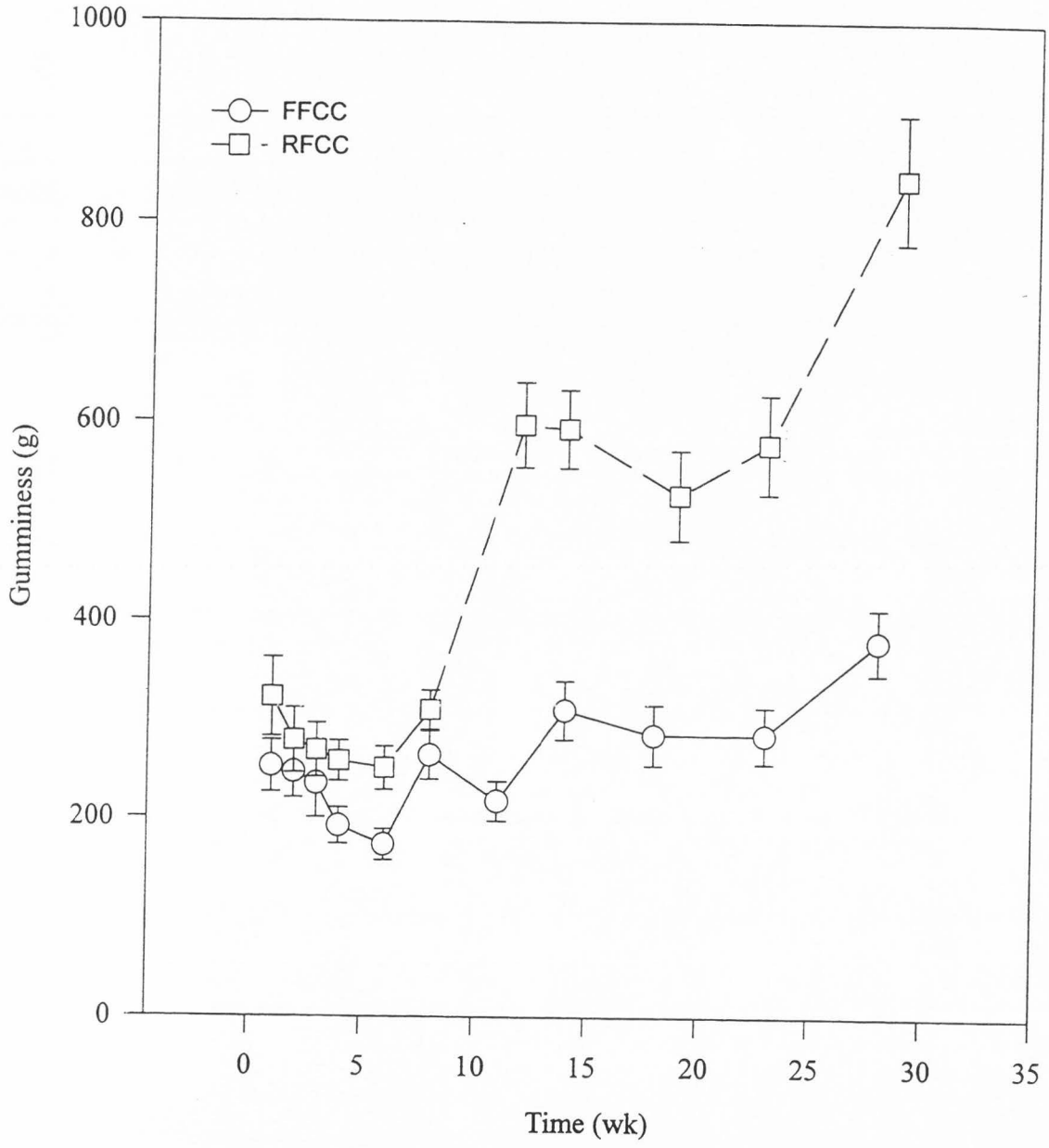

Fig. 5.3--Change in gumminess of FFCC and RFCC during ripening.

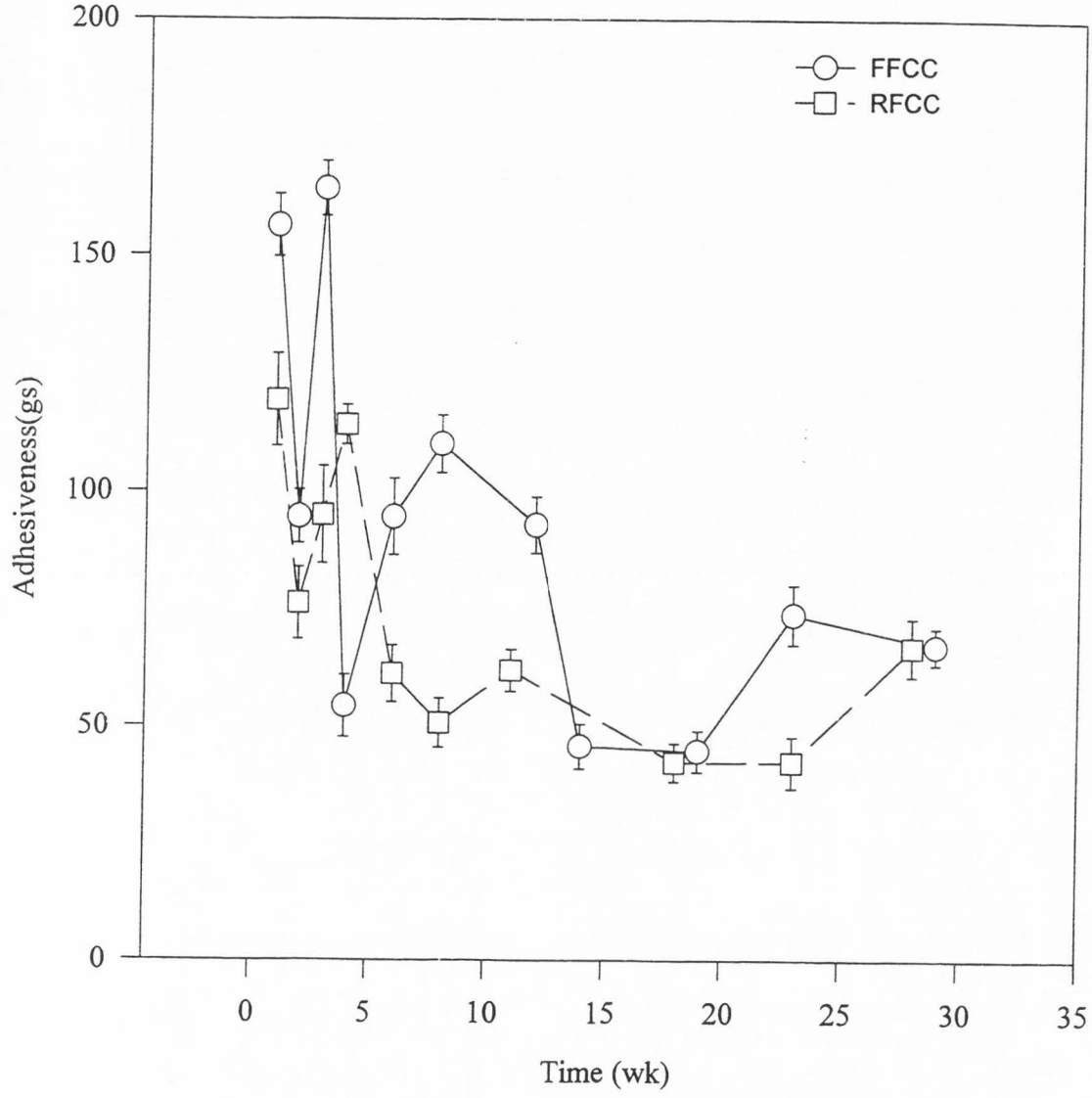

Fig. 5.4--Change in adhesiveness of FFCC and RFCC during ripening.

The increased protein content altered the protein matrix, making it more compact and, therefore, less adhesive, alternatively more cohesive. Both RFCC and FFCC ripened for 6 months were less adhesive (Fig. 5.4). A similar trend was observed by Bryant et al. (1995) in their study of low-fat cheeses wherein the authors reported lower values for adhesiveness in cheese ripened for 4 months. This can be attributed to the fact that during ripening, in addition to proteolysis, there is also a movement of moisture and a subsequent rearrangement of the protein matrix. More homogeneous regions are found in the matrix, resulting in a decrease in adhesiveness with time. Protein content was the dominant factor influencing adhesiveness of cheese with varying composition (Chen et al., 1979).

Springiness is the rate and extent that a deformed material goes back to its undeformed state after the force is removed. Bryant et al. (1995) observed that reduced-fat cheeses (13-27% fat content) were springier than full-fat (32-34%) cheese using a 55% compression test. Emmons et al. (1980) demonstrated that low-fat Cheddar cheese was springier than full-fat Cheddar cheese, thereby hypothesizing that reducing fat content resulted in fewer fat globules with more casein present per unit volume as evidenced by electron micrographs. In this research, the mean values of springiness determined using texture profile analysis are not significantly different between FFCC (31.3% fat content) and RFCC (21.4% fat content) (Fig. 5.5). A plausible reason could be that the 20% deformation used in the TPA experiment is not sufficient. At 20% deformation, perhaps the fat and protein present are mechanically exerted within their elastic limit and, irrespective of the fat content, have a similar behavior. However, when

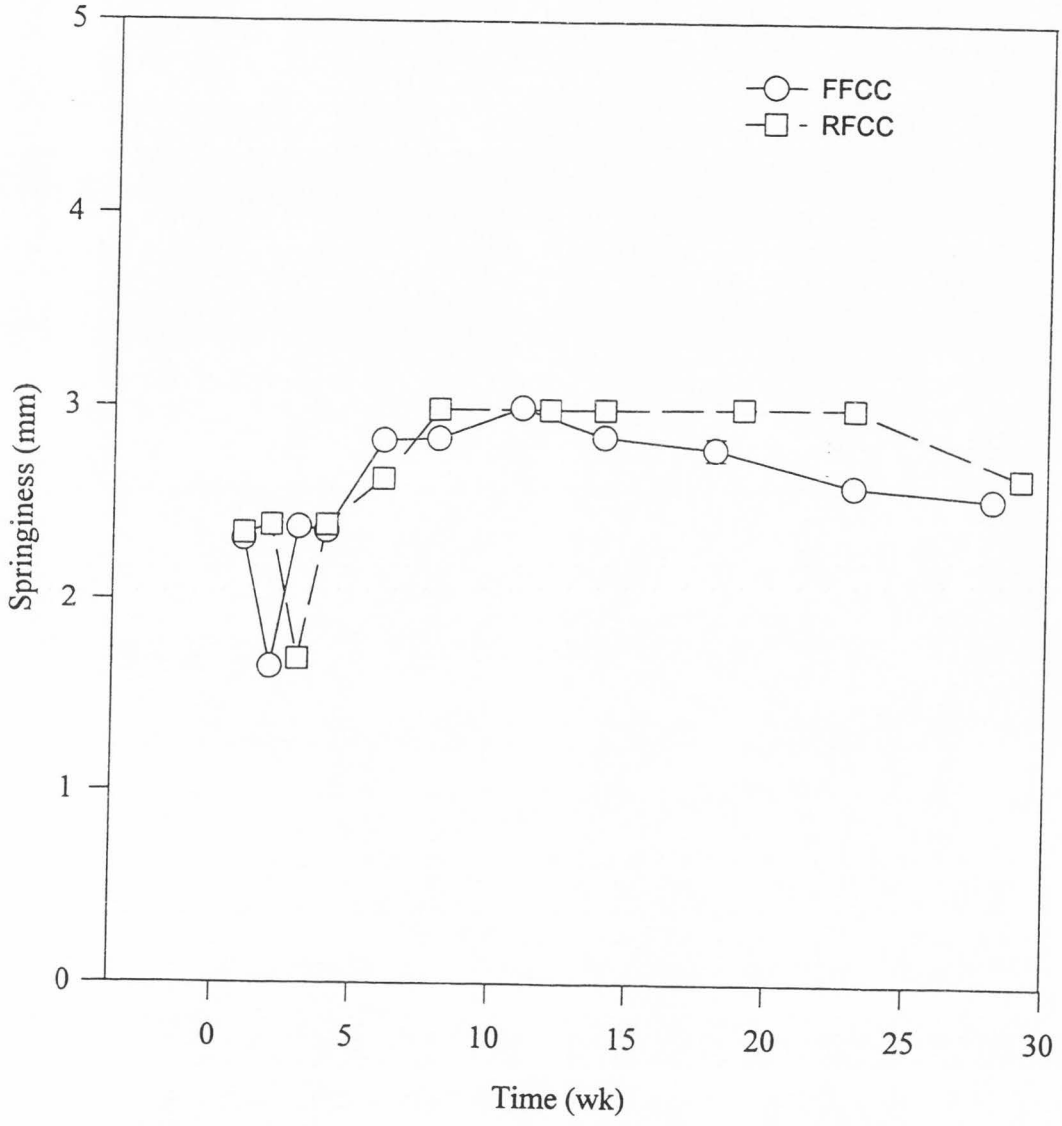

Fig. 5.5--Change in springness of FFCC and RFCC during ripening.

higher deformation values are used, the behavior of these constituents extends to the plastic state, and fat being more pliable than protein tends to impart a higher degree of springiness to higher fat cheeses. A slight increase in springiness was observed with ripening for both FFCC and RFCC. This may be due to the fact that moisture available for the casein matrix is restricted due to the hydration of peptide and hydrolysis products formed from proteolysis. In addition, protein level is the dominant component affecting the elasticity in cheese (Chen et al., 1979; Emmons et al., 1980; Bryant et al., 1995). Tunick et al. (1991) reported greater values for springiness with reduced moisture levels in Mozzarella cheese. Higher moisture in reduced-fat Cheddar cheese did not decrease its springiness. Hence, chewiness, the product of hardness, cohesiveness, and springiness, increased with a decrease in fat content and an increase in aging time, and followed the same trend of hardness, as shown in Fig. 5.6.

Correlation of TPA and FTIR data

Texture profiles of full-fat and reduced-fat Cheddar cheese (FFCC and RFCC) were correlated with the absorbance of peak areas of main reactive groups (Tables A3 and A4). Hardness is more related to the change in absorbance of bands at 1167, 1461, 1744, and 2850 cm^{-1} for FFCC (Table 5.2), and at 1116, 1640-1650, 1744, and 2850 cm^{-1} for RFCC (Table 5.3) as seen from their respective correlation coefficients. The difference in hardness is highly related to the band at 1167 cm^{-1} arising from C-O stretch for FFCC instead of the band at 1640-1650 cm^{-1} arising from protein amide I and bound water (Surewicz and Mantsch, 1988; van de Voort et al., 1992) for RFCC. During cheese ripening, fat content of cheese, hydrolysis products of proteolysis, and water available for

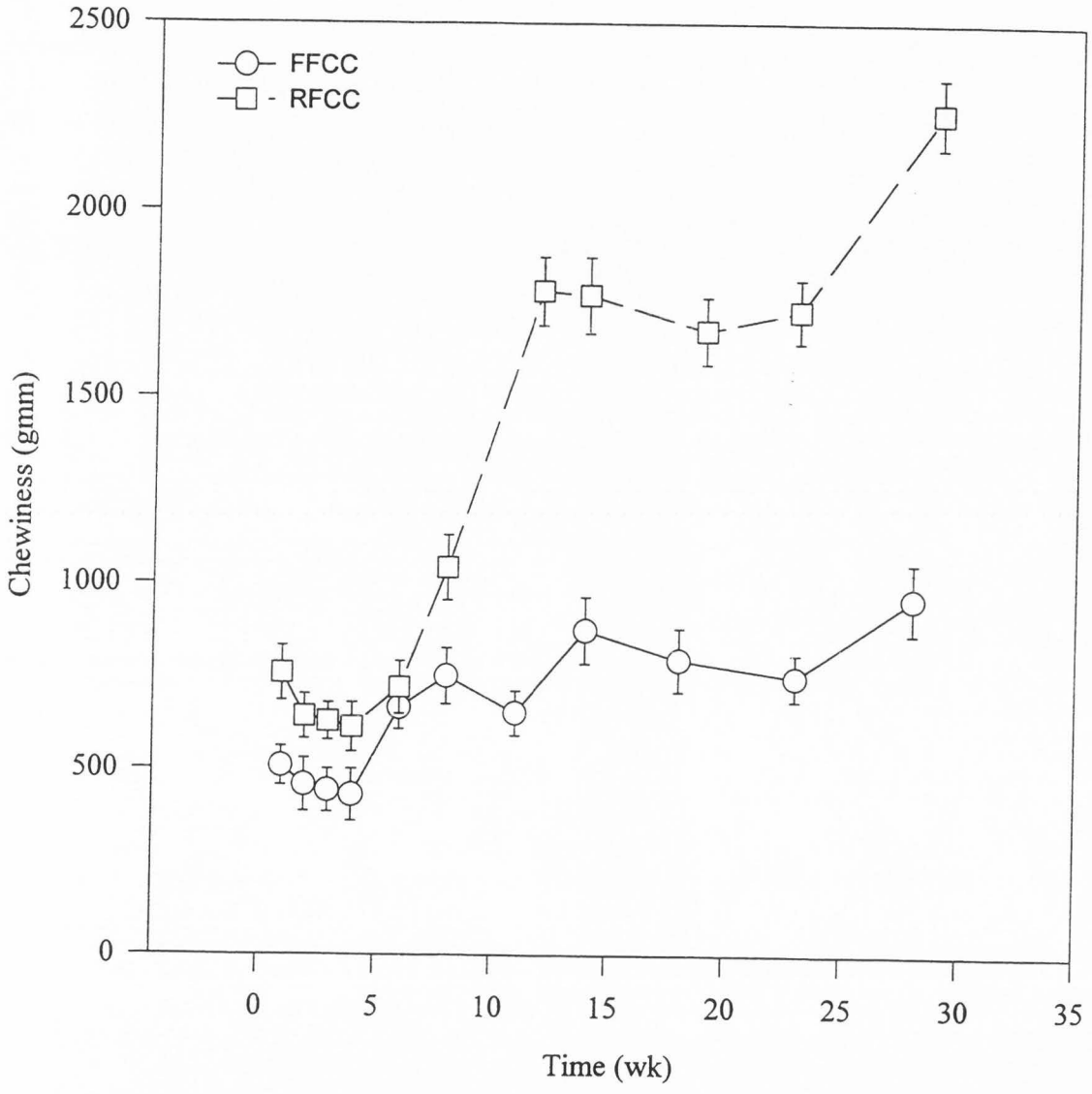

Fig. 5.6--Change in chewiness of FFCC and RFCC during ripening.

Table 5.2--Correlation coefficients for hardness versus absorbance of peak area of each functional group for FFCC

Frequency of peak (cm^{-1})	Related functional groups	Correlation coefficient
1116	C-N & C-C stretch	-0.16
1167	C-O stretch	0.66
1240	C-C & C-N stretch	0.43
1461	$-CH_2X$, X=CO, O, N, OCO	0.62
1744	R(CO)X, X= O^-, OR, H, R	0.60
2850	$R(CH_2)R$	0.44
2930	CH_3R	0.29
1530-1540	amide I (peptide bond)	0.16
1640-1650	random portion	0.17
1653-1658	helical portion	-0.03

Hardness = 248.61 + 14.56 * 1116cm^{-1} + 71.83 * 1167cm^{-1} - 92.26 * 1240cm^{-1}
+ 22.45 * 1461cm^{-1} - 7.36 * 1744cm^{-1} + 2.10 * 2850cm^{-1}
- 5.91 * 2930 cm^{-1} - 4.03 * 1640-1650 cm^{-1}

R^2: 0.67

Root MSE: 87.91

Table 5.3--Correlation coefficients for hardness versus absorbance of peak area of each functional group for RFCC

Frequency of peak (cm^{-1})	Related functional groups	Correlation coefficient
1116	C-N & C-C stretch	-0.25
1167	C-O stretch	-0.05
1240	C-C & C-N stretch	-0.09
1461	$-CH_2X$, X=CO, O, N, OCO	-0.10
1744	R(CO)X, X= O^-, OR, H, R	0.26
2850	$R(CH_2)R$	-0.28
2930	CH_3R	-0.16
1530-1540	amide I (peptide bond)	-0.05
1640-1650	random portion	0.32
1653-1658	helical portion	-0.06

Hardness = 591.31 - 55.87 * 1116cm^{-1} - 92.20 * 1240cm^{-1} - 18.69 * 1461cm^{-1} + 70.43 * 1744cm^{-1} - 7.03 * 2850cm^{-1} - 27.10 * 2930cm^{-1} + 68.04 * 1640-1650 cm^{-1}

R^2: 0.51

Root MSE: 214.96

solvation of protein chains all contribute to the development of hardness (Lawrence and Gilles, 1987). Change in absorbance of the amide I band is more significantly correlated to RFCC, which led us to hypothesize that the function of protein is a predominate factor affecting hardness during ripening. In FFCC, a more significant correlation between hardness and the band at 1167 cm^{-1} corresponding to fat and protein content showed that lipolysis and proteolysis significantly contributed to hardness in FFCC during aging. The effect of lipolysis and its subsequent contribution to hardness is due to a 10% increase in the fat content in FFCC. A maximum R^2 obtained by regressing hardness with predominant reactive groups in FFCC is 0.67 and that for RFCC is 0.51.

Springiness has a significant correlation with the bands at 1744, 1167, and 2850 cm^{-1} for FFCC (Table 5.4), and at 1116 and 1744 cm^{-1} for RFCC (Table 5.5). Protein bands that changed greatly during aging had lower correlation with springiness. A maximum R^2 of 0.75 for FFCC and 0.54 for RFCC is obtained through regression analysis. The primary groups correlating with springiness are R(CO)X (X= O^-, OR, H, R) and C-O stretch in fat and protein for FFCC, and R(CO)X (X= O^-, OR, H, R) and C-N and C-C stretch for RFCC. Change in absorbance of these bands represented the rate of lipolysis and proteolysis and its product in cheese. Hence, major factors that affect springiness or elasticity of cheese are fat and protein content in cheese, status of moisture, and the rate of lipolysis and proteolysis.

Tables 5.6 and 5.7 show the correlation of adhesiveness to the reactive groups in the FTIR spectra. The highly correlated bands for FFCC are at 1167, 1744, and 1530-

Table 5.4--Correlation coefficients of springiness versus absorbance of peak area of each functional group for FFCC

Frequency of peak (cm^{-1})	Related functional groups	Correlation coefficient
1116	C-N & C-C stretch	-0.21
1167	C-O stretch	0.62
1240	C-C & C-N stretch	0.46
1461	$-CH_2X$, X=CO, O, N, OCO	0.37
1744	R(CO)X, X= O^-, OR, H, R	0.70
2850	$R(CH_2)R$	0.53
2930	CH_3R	0.26
1530-1540	amide I (peptide bond)	0.17
1640-1650	random portion	0.25
1653-1658	helical portion	0.31

Springiness = 1.36 + 0.02 * 1167cm^{-1} + 0.09 * 1240cm^{-1} - 0.22 * 1461cm^{-1}
+ 0.23 * 1744cm^{-1} - 0.001 * 2850cm^{-1} - 0.06 * 2930cm^{-1}
- 0.08 * 1530-1540 cm^{-1} + 0.02 * 1640-1650 cm^{-1}

R^2	0.75
Root MSE:	0.35

Table 5.5--Correlation coefficients of springiness versus absorbance of peak area of each functional group for RFCC

Frequency of peak (cm^{-1})	Related functional groups	Correlation coefficient
1116	C-N & C-C stretch	-0.33
1167	C-O stretch	-0.11
1240	C-C & C-N stretch	-0.01
1461	$-CH_2X$, X=CO, O, N, OCO	-0.12
1744	R(CO)X, X= O^-, OR, H, R	0.33
2850	$R(CH_2)R$	0.05
2930	CH_3R	-0.11
1530-1540	amide I (peptide bond)	-0.05
1640-1650	random portion	0.14
1653-1658	helical portion	-0.09

Springiness = 2.89 - 0.20 * 1116 cm^{-1} - 0.25 * 1167 cm^{-1} + 0.16 * 1461cm^{-1}
+ 0.18 * 1744 cm^{-1} - 0.05 * 2930cm^{-1}
- 0.04 * 1658 cm^{-1} + 0.003 * 1640-1650 cm^{-1}

R^2: 0.54

Root MSE: 0.36

Table 5.6--Correlation coefficients of adhesiveness versus absorbance of peak area of each functional group for FFCC

Frequency of peak (cm^{-1})	Related functional groups	Correlation coefficient
1116	C-N & C-C stretch	0.09
1167	C-O stretch	0.28
1240	C-C & C-N stretch	0.12
1461	$-CH_2X$, X=CO, O, N, OCO	-0.12
1744	R(CO)X, X= O^-, OR, H, R	0.24
2850	$R(CH_2)R$	0.11
2930	CH_3R	0.09
1530-1540	amide I (peptide bond)	-0.21
1640-1650	random portion	-0.11
1653-1658	helical portion	0.13

Adhesiveness =86.37 + 45.79 * 1167 cm^{-1} - 48.72 * 1240 cm^{-1} - 21.84 * 1461cm^{-1} - 3.09 * 1744 cm^{-1} - 1.37 * 2850cm^{-1} + 5.26 * 1530-1540 cm^{-1} - 1.27 * 1640-1650 cm^{-1} + 1.83 * 1658 cm^{-1}

R^2:	0.54
Root MSE:	34.69

Table 5.7--Correlation coefficients of adhesiveness versus absorbance of peak area of each functional group for RFCC

Frequency of Peak (cm^{-1})	Related Functional Groups	Correlation Coefficient
1116	C-N & C-C stretch	0.19
1167	C-O stretch	-0.08
1240	C-C & C-N stretch	-0.19
1461	$-CH_2X$, X=CO, O, N, OCO	-0.23
1744	R(CO)X, X= O^-, OR, H, R	0.25
2850	$R(CH_2)R$	-0.04
2930	CH_3R	-0.09
1530-1540	amide I (peptide bond)	-0.22
1640-1650	random portion	0
1653-1658	helical portion	-0.20

Adhesiveness =73.60 + 13.98 * 1116 cm^{-1} + 35.23*1167 cm^{-1} - 103.55*1240 cm^{-1}
- 13.33 * 1461 cm^{-1} + 10.29 * 1744cm^{-1} + 5.30 * 2930 cm^{-1}
- 6.36 * 1653-1658 cm^{-1} + 12.48 * 1530-1540 cm^{-1}

R^2: 0.59

Root MSE: 26.71

1540 cm^{-1} (Table 5.6), and those for RFCC are at 1461, 1744, 1530-1540 cm^{-1} (Table 5.7). Protein content in cheese is the dominant factor influencing adhesiveness of cheese with varying composition; however, the movements of moisture and rearrangement of protein matrix during ripening also affect adhesiveness of cheese. Both fat- and protein-related bands correlate with adhesiveness but have very low correlation coefficients. Maximum R^2 is 0.54 for FFCC and 0.58 for RFCC.

Higher values of R^2 could be expected if larger sample data set and longer aging times are used to set up the model. Nonlinear statistical analysis and incorporation of interactions in the model may also improve the correlation. The spectra data may not completely reflect the complex physical and biochemical changes during cheese aging. However, the work presented is a first attempt to characterize cheese texture with respect o the change in chemical groups from FTIR spectra.

CONCLUSION

Hardness of full-fat and reduced-fat Cheddar cheese during aging had a similar trend. Hardness decreased within 4 to 6 weeks and increased after that. Greater hardness for RFCC than that for FFCC was due to the effect of reduction in fat content, function of protein, and moisture available for solvation of the protein chain due to its attachment to the cleaved ionic groups during proteolysis.

Adhesiveness decreased with a reduction of fat content and increasing ripening time. More homogeneous regions were found in the matrix, which resulted in a decrease in adhesiveness with time. Protein content was the dominant factor influencing

adhesiveness of cheese with varying composition. Cohesiveness increased slightly with a decrease in fat content but did not change significantly during aging. Removal of fat, which increased protein content, altered the protein matrix, making it more compact and, therefore, less adhesive, alternatively more cohesive. Springiness was not significantly different between FFCC (31% fat) and RFCC (21% fat) but increased slightly during aging. Gumminess, a product of hardness and cohesiveness, and chewiness, a product of hardness, cohesiveness, and springiness, followed the same trend as hardness.

Correlation of texture and spectra data varied depending on the effect of specific bands in spectra on the texture parameters. A maximum R^2 obtained for hardness in FFCC was 0.67 and that for RFCC was 0.51. R^2 for springiness was 0.75 for FFCC and 0.54 for RFCC, and that for adhesiveness was 0.54 and 0.59 for FFCC and RFCC, respectively. A higher R^2 value could be expected if a larger sample set and longer aging time are used to set up the model. Nonlinear statistical analysis and incorporation of interactions in the model may also improve the correlation. The work presented is a first attempt to characterize cheese texture with respect to the change in chemical groups from FTIR spectra. The work conducted and further improvement will provide a chemical basis to explain a mechanical phenomenon.

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CHAPTER 6

SUMMARY

Well-separated spectra of cheeses were obtained by using a microtome sampling technique at a resolution of 4 cm^{-1}, using 32 scans/sample, and a sample thickness of 16 μm. Moisture in the sample was critical for a satisfactory performance using this measurement technique. Repeatable spectra could be obtained after equilibrating the sample for at least 10 min. Sample inhomogeneity and the manner of application are main factors that affect the accuracy and reproducibility of spectra. However, this could be minimized by taking samples from different locations in the cheese and improving the attachment of the sample to the silver chloride crystal. The absorbance intensity of fat- and protein-related bands was proportional to the fat and protein contents in the cheese sample. FTIR spectroscopy coupled with the microtome sampling accessory could also be used to monitor changes in the secondary structure of casein protein due to proteolysis during ripening.

The spectra of FFCC and all the RFCC (25%, 50%, and 75%) were collected during their aging. Correlation of the absorbance of protein- and fat-related bands with proximate analysis resulted in an R^2 of 0.77 for fat and 0.86 for protein-related bands, respectively. The NPN content was determined, and the absorbance at 210 nm due to α_{s1}-casein was measured using CE experiments. Comparison of the 90-days-old with that of a 1-day-old sample indicated that the breakdown of α_{s1}-casein by chymosin present in the coagulant resulted in a 3.2-fold increase in α_{s1}-casein f24-199 and a 4.4-fold increase in

α_{s1}-casein f102-199 fragments during aging. As a result of the increase of small fragments, NPN content was increased during aging for both FFCC and RFCC.

Absorbance of fat-related bands at 1167, 1744, 2850, and 2930 cm^{-1}, and protein-related bands in the range of 1500-1580 and 1600-1690 cm^{-1} changed greatly during ripening predominantly due to proteolysis and lipolysis. Relatively, absorbance at bands from 1100-1240 cm^{-1} related to both fat and protein contents changed slightly, which could be suitable for quantitative analysis of fat and protein contents in cheese.

Variation in the bands at 1620-1635, 1640-1648, 1650-1558, and 1660-1688 cm^{-1} assigned to β-sheet, random, helix, and the turns/sheet portion of secondary structure of protein, respectively, was observed for RFCC and FFCC during ripening. The predominance of the bands at 1650-1658 and 1660-1688 cm^{-1} due to the helical and turns/sheet portion of secondary structure indicated that these structures were more stable than other secondary structures found in protein. Absorbance change of these bands could be due to the formation of various small polypeptides produced during the breakdown of casein by rennet and proteinases during ripening of 1 to 29 weeks. The analysis results indicated that different polypeptides, small peptides and segments, and free amino acids were formed during the breakdown of casein by proteolysis in each cheese during the cheese-making and ripening stages. Since the rate and products of lipolysis and proteolysis are important for flavor and texture development, this information could help us to understand the biochemical reactions and to monitor cheese maturation.

A simple regression model for ripening index was obtained by correlation of the absorbance of key reactive groups with ripening time. A maximum R^2 of 0.83 for FFCC

and 0.59 for RFCC was obtained for the respective ripening index regression models. This methodology could be adopted to study the ripening of new cheese varieties using accelerated ripening methods. The models could be improved if more samples could be obtained and data collected beyond 28 weeks of ripening.

The change in texture (hardness, gumminess, chewiness, springiness, adhesiveness, and cohesiveness) and its relation to the change in reactive groups was also studied. Higher hardness for RFCC than that for FFCC was due to the effect of reduction in fat content, function of protein, and moisture available for solvation of the protein chain due to its attachment to the cleaved ionic groups during proteolysis. Adhesiveness decreased with a reduction of fat content and an increase in ripening time. Protein content was the dominant factor influencing adhesiveness of cheese with varying composition. Cohesiveness increased slightly with a decrease in fat content but did not change significantly during aging. Removal of fat, which increased protein content, altered the protein matrix, making it more compact and, therefore, less adhesive, and alternatively more cohesive. Springiness was not significantly different between FFCC (31% fat) and RFCC (21% fat) but increased slightly during aging. Gumminess, a product of hardness and cohesiveness, and chewiness, a product of hardness, cohesiveness, and springiness, followed the same trend as hardness.

Correlation of texture and spectra data varied depending on the effect of specific bands in spectra on the texture parameters. A maximum R^2 obtained for hardness in FFCC was 0.67 and that for RFCC was 0.51. R^2 for springiness was 0.75 for FFCC and 0.54 for RFCC, and that for adhesiveness was 0.54 and 0.59 for FFCC and RFCC,

respectively. A higher R^2 value could be expected if a larger sample set and longer aging time are used to set up the model. Nonlinear statistical analysis and incorporation of interactions in the model may also improve the correlation.

APPENDICES

Appendix A

Tables

Table A.1--Schedule for making FFCC (USU dairy lab)

Steps in making	Time	Acid(%)	content
Add starter		0.16	0.7-0.8%
Add rennet	0:00	0.16	90 ml/1000#
Stir			
Set			
Cut	0:30	0.10	
Start heat	0:45	0.10	
Stop heat	1:15	0.11	38.5°C
Start dipping	2:15	0.12	
End Dipping	2:30	0.14	
Pack	2:45	0.18	
Pile 2 high	3:30	0.28	
Pile 3 high	4:00	0.35	
Mill	4:30	0.40-0.45	pH = 5.4
1st salt application	4:40		
2nd salt application	4:50		
3rd salt application	5:00		
Hoop	5:10		
Press	5:30	50-60 psig	

Table A.2--Schedule for making RFCC (Wisconsin center for dairy research)

Steps in making	Time	pH	content
Add starter	0.00	6.60^{m}	1.25% CTD
Add $CaCl_2$	0:15		3.0 ounces/1000#
Add Rennet	0:20	6.54^{m}	2.25 ounces/1000#
Cut	1:10	6.53^{w}	
Start heat	1:20		
Reach cook temp and drain	1:45	6.48^{w} 38.5°C	
Cut curd mass and cheddar	2:05	6.28^{c}	
Cheddar 2 high	2:30	6.20	
Mill curd	4.05	5.95^{c}	
Salt curd	4:20		0.275%
Hoop	4:40		
Press	4:55		

(m = milk pH, w = whey pH, c = curd pH)

Table A.3--Data for absorbance at function group related bands in FFCC during aging

Time	1116cm	1167cm	1240cm	1461cm	1744cm	2850cm	2930cm	1529cm	1640cm	1658cm
1	0.6	1.85	0.8	1.29	3.45	8.4	2.68	0.185	0.29	0.75
2	0.37	5.71	3.05	0.46	9.68	29.54	19.1	0.46	0.57	2.87
3	1.12	2.18	1.5	0.23	4.95	3.7	4.57	0.21	0.14	0.57
6	1.51	4.28	2.06	0.34	7.17	30.55	6.46	0.2	0.76	1.99
10	0.48	8.34	3.51	5.71	13.57	51.61	22.91	11.21	29.53	0
14	2.02	5.76	2.45	3.87	11.13	10.38	10.96	1.75	0.37	1.31
18	1.97	4.67	2.35	3.3	10.2	9.83	9.06	1.56	0.31	1.64
23	0.3	7.95	3.3	5.13	14.75	39.3	20.8	2.26	0.42	1.72
27	0.17	7.2	3.1	4.77	13.04	30.4	12	2.16	0.89	1.85
1	0.6	4.14	1.89	0.39	9.15	31.42	11.21	0.24	0.68	2.4
2	1.3	2.52	1.22	1.86	4.59	21.6	4.4	3.26	0	1.04
3	2.19	5.57	3.04	0.72	8.98	25.94	18.81	0.7	0.78	1.96
4	1.3	2.33	1.48	0.23	5.52	18.3	5.14	0.17	0.06	1.31
6	0.45	3.69	2.59	0.35	6.69	35.7	5.16	0.19	0.07	1.99
11	0.1	4.47	2.24	3.21	15.96	23.17	10.23	5.65	0	12.97
14	0.39	5.84	3	4.43	10.23	41.22	13.15	9.28	18.6	0
18	0.02	6.45	2.99	4.73	12.68	35.22	11.06	1.98	0.83	2.11
23	0.09	4.33	2.15	2.94	8	16.76	11.04	0.16	0.47	1.47
28	1.56	4.34	2.26	2.95	9.35	19.53	13.67	1.48	0.43	1.36

Table A.4--Data for absorbance at function group related bands in RFCC during aging

Time	1116cm	1167cm	1240cm	1461cm	1744cm	2850cm	2930cm	1529cm	1640cm	1658cm
1	2.44	2.13	1.32	0.18	3.83	9.32	1.88	0.1	0.34	2
2	3.02	1.96	1.4	0.2	3.81	15.19	6.22	0.16	0.13	0.48
4	1.61	2.68	2.28	0.09	7.42	7.89	10.85	0.88	1.03	0.29
8	0.8	3.41	2.62	0.26	8.61	35.2	11.55	0.21	0.16	3.4
14	0.98	1.66	1.3	2.94	2.47	7.53	6.42	3.01	6.31	0
19	0.73	0.71	0.74	0.15	2.64	9.86	1.26	0.15	0.08	0.41
23	0.96	1.47	1.4	0.14	4.4	1.5	5.21	0.1	0.04	1.44
29	0.56	3.01	2.15	0.24	6.95	4.27	7.69	1.57	0.41	2.75
1	1.78	1.88	1.04	1.23	3.07	5.67	5.87	0.05	0.45	1.31
2	3.47	0.98	1.1	0.98	1.62	21.07	2	0.67	0.66	0.49
3	0.42	2.27	1.41	1.99	4.13	25.27	7.08	5.56	0	14.14
4	0.86	1.55	1.06	0.11	3.86	4.13	4.45	1.02	0.28	1.18
6	0.47	1.55	1.04	0.12	3.69	11	5.46	0.2	0	1.31
10	1.25	1.9	1.52	1.41	4.47	6.3	5.51	3.13	0.3	1.24
14	2.04	2.15	1.8	1.88	5.17	11.71	9.15	0.63	0.26	1.38
18	2.71	3.16	2.33	0.33	6.52	10.11	2.85	1.12	0.89	1.14
23	1.26	1.28	1.03	1.11	3.47	12.98	3.67	0.23	0	1.08
27	0.08	2.17	1.68	0.17	5.4	6.82	7.93	0.18	0.2	1.88

Appendix B

Figures

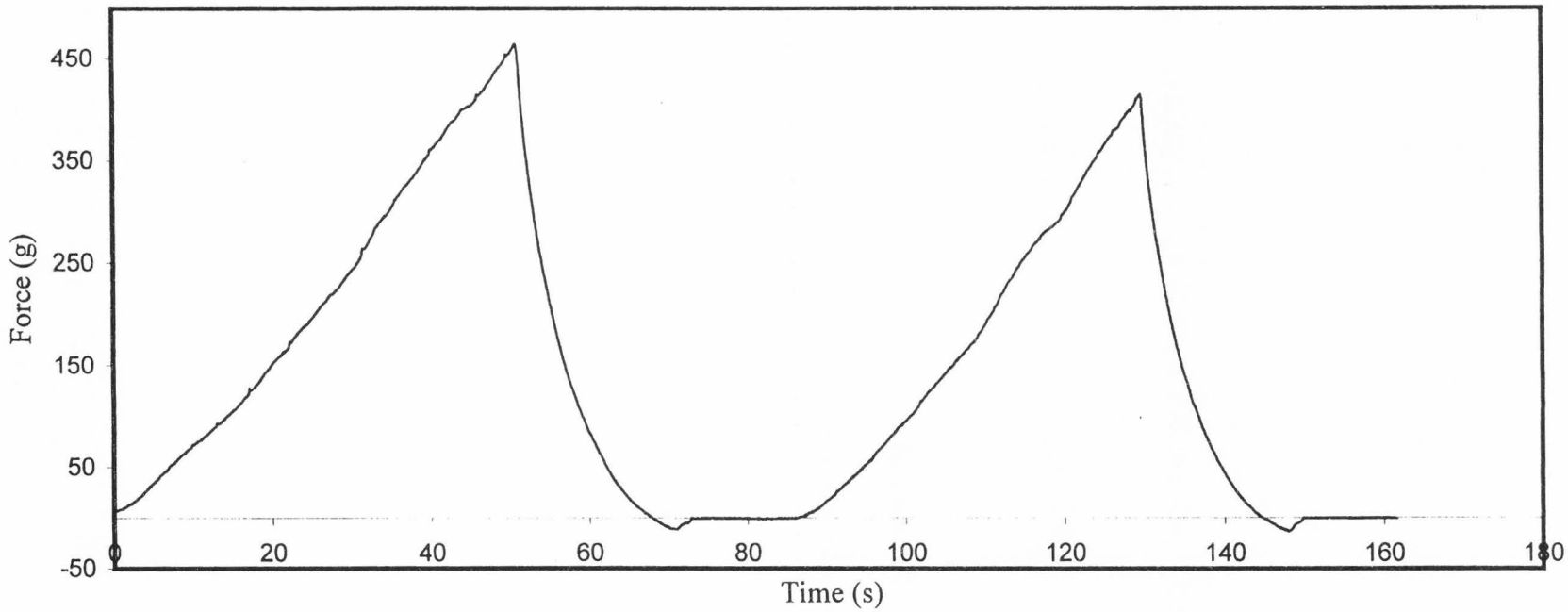

Fig. B1--Typical curve of TPA analysis for RFCC

Appendix C

Permission Letter

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