Monitoring Composition and Flavor Quality of Cheddar Cheese during Ripening Using a Rapid Spectroscopic Method

Anand Subramanian*

Introduction

About 9.13 billion pounds of cheese is produced in the US every year, of which 34% is Cheddar cheese (NASS, 2007). Cheddar cheese composition and flavor quality, which influence the consumer acceptance, price and food processing application, develop during the ripening process. Cheese ripening or maturation is a slow process (2-24 months) characterized by series of complex physical, chemical, and microbiological changes affecting the principal components of the cheese (Singh et al., 2003). The complex nature of the cheese ripening process and heterogeneous nature of cheese make it a challenge to produce cheese of uniform composition and sensory properties, especially flavor.

The effect of the manufacturing process, the composition of milk (such as protein and fat level), and the biochemical events that occur during ripening will influence the composition and final quality of the cheese (Chen et al., 1998). Furthermore, the composition and flavor profile of cheese are complex and variety- or type-specific (Akalin et al., 2002) and are determined to a great extent by breakdown of proteins, fats and carbohydrates during ripening (Singh et al., 2003; Bachmann et al., 1999). The principle compounds that contribute to the flavor include organic acids, amino acids, sulfur compounds, lactones, methyl ketones, alcohols and phenolic substances (Seitz, 1990; Urbach, 1993). Currently, cheese components such as fat, moisture, pH and salt content are determined using standard chemical and chromatographic methods and flavor by trained taste panels. These approaches are complex, labor-intensive, expensive and

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time-consuming, thereby hindering quality control efforts. Hence, there is a need for rapid and reliable instrumental methods for simultaneous determination of composition and flavor quality of cheese. A rapid method apart from saving time and money for the cheese industry will also help in ensuring better product quality and safety and understanding cheese ripening.

Cheddar cheese flavor is due to a balance of concentration and ratios of various classes of compounds (Kosikowski and Mucquot, 1958; McSweeney and Sousa, 2000). Hence, an instrumental method that is capable of simultaneous monitoring of multiple compounds and their functional groups is necessary to determine flavor quality. Attempts have been directed at finding and evaluating microbiological and biochemical parameters by which cheese could be classified and whereby uniform cheese quality could be established (Singh et al., 2003; Adda et al., 1982; Farkye and Fox, 1990; Seitz, 1990). Methods such as high performance liquid chromatography (Lues and Bekker, 2002; Akalin et al., 2002), gas chromatography (Partidario et al., 1998; Thierry et al., 1999) and mass spectrometry (Alli et al., 1998) have been investigated for analysis of cheese components. These studies have made significant contributions to the understanding of the ripening process. However, they are complicated, time-consuming, require different conditions and accessories for analyzing different class of compounds and have limited applications as routine quality control methods. Due to these reasons, no reliable instrumental methods exist for rapid analysis of flavor quality.

Fourier transform infrared (FT-IR) spectroscopy is a simple, rapid and reliable technique that has been widely researched and applied for analysis of food components. It is based on the principle that different chemical functional groups require different amounts of energy (different wavelengths) for excitation. FT-IR spectroscopy monitors the absorbance of infrared light by functional groups in the sample to provide a spectrum (chemical profile) of the sample. A

Fourier transform mid-infrared (4000 to 400 cm-1) spectrum shows the overall chemical composition of the sample. FT-IR spectroscopy combined with multivariate analysis has been suggested for rapid analysis of cheese by many researchers. This technique has been applied to compositional analysis including fat, protein, and moisture in cheese (Rodriguez-Saona et al., 2006; Chen and Irudayaraj, 1998; Chen et al., 1998; McQueen et al., 1995). However, analysis of cheese flavor by spectroscopy and simultaneous analysis of cheese composition and flavor quality has not been explored. This is mainly because of difficulties in the sampling procedures and heterogeneous nature of cheese (McQueen et al., 1995). Furthermore, interference from other cheese constituents is a challenge that needs to be overcome for efficient FT-IR analysis. Hence, an effective sample preparation method is essential for analysis of flavor compounds in cheese. The objectives of this research were to 1) develop a suitable sample preparation and FT-IR technique that would enable simultaneous analysis of cheese composition and flavor quality and 2) develop multivariate statistical models using the spectra to determine composition and flavor quality of Cheddar cheese. To the best of our knowledge this is the first research on rapid and simultaneous analysis of composition and flavor quality of cheese using FT-IR spectroscopy.

Material and Methods

Cheddar Cheese Samples

Twelve different Cheddar cheese samples ripened for a period of 73 days were provided by a commercial cheese manufacturer. Samples were collected on days 7, 15, 30, 45 and 73 during ripening, vacuum packaged and stored at -40°C until analysis.

Determination of Cheese Composition and Quality

Moisture, fat, salt content and pH of the samples were determined by the manufacturer using methods established and approved by Association of Official Analytical Chemists (AOAC). The sensory flavor quality of these cheeses were analyzed by trained quality assurance personnel in the production facilities and provided along with the cheese samples. The organic acid content of the cheese samples were determined by reverse-phase high performance liquid chromatograph (HPLC) (HP 1050, Agilent Technologies, Santa Clara, CA) equipped with a Prevail™ organic acid column (Alltech Associates Inc., Deefield, IL) and a UV detector. The amino acids present in the samples were determined by gas chromatograph (Agilent 6890, Agilent Technologies, Santa Clara, CA) fitted with a Hexiflex® amino acid capillary column (Alltech Associates Inc., Deefield, IL) and a flame ionization detector.

Sample Preparation and FT-IR Analysis

The cheese samples were cryogenically ground into their powders using liquid nitrogen. These powders were then sequentially extracted with organic solvents to extract essential components from the cheese with little or no interfering material. FT-IR spectroscopy of the extracts was carried out on a Varian 3100 FT-IR spectrometer (Varian Inc., Palo Alto, CA) equipped with PERMAGLOW™ mid-IR source, extended range potassium bromate infrared beam splitter and deuterated triglycine sulfate detector. Aliquots (10 µL) of the extracts were placed on a 3-bounce MIRacle™ attenuated total reflectance (ATR) accessory with an zinc selenide crystal (Pike Technologies, Madison, WI) and vacuum dried to form a thin film. Infrared spectra were recorded in the mid-infrared region, between the wavenumbers 4000 and 700 cm^{-1}, using the data collection software (Varian Resolutions Pro v4.05, Varian Inc., Palo Alto, CA). In order to improve the signal to noise ratio 64 scans were averaged for each spectrum. For each cheese, three samples were collected from different locations and powdered.

Five independent extractions were made for each powder and 3 spectra were collected per extract to yield at least 15 spectra per cheese sample and a total of at least 900 spectra for the whole study.

Multivariate Analyses

Multivariate analyses of the data were carried out using a commercially available comprehensive chemometrics modeling software called Pirouette® (v3.11, Infometrix Inc., Woodville, WA). For analysis, spectra were imported into Pirouette®, mean-centered, transformed into their second derivative using a Savitzky-Golay polynomial filter (five-point window) and vector-length normalized. The FT-IR spectra of the samples were matched with their age, composition and quality data to develop prediction models. Partial Least Squares Regression (PLSR) models with cross-validation were developed to predict the age, moisture, fat, pH, salt, organic and amino acid contents of the samples. Soft independent modeling of class analogy (SIMCA) classification models were developed to classify cheese based on flavor. The data were projected onto the first three principal component axes to visualize clustering of samples in 3D space based on their flavor quality (fermented, sour, good cheddar, etc.). The spectral regions influencing the classification of the cheeses were determined from the measure of variable importance (discriminating power). The some of the general biochemical changes occurring during Cheddar cheese ripening leading to the formation of “Good Creamy Cheddar” flavor were identified.

Results and Discussion

Extraction with organic solvents enabled removal compounds that interfered with the detection of essential compounds such organic acids and amino acids that contribute to the flavor. The resulting cheese extract contained organic acids, short chain fatty acids and their

esters, alcohols, amino acids, and small peptides, all of which are known to contribute significantly to cheese flavor (Singh et al., 2003; Seitz, 1990; Urbach, 1993). FT-IR spectra of the extracts were collected in the mid-infrared region (4000-700 cm^{-1}), using a 3-bounce zinc selenide crystal. In a 3-bounce FT-IR crystal, infrared light bounces on the sample 3 times, increasing the absorbance and hence the signal. Drying of the extract on the crystal resulted in the formation of a uniform film of sample. The drying time per sample was 3 min. The sample preparation method allowed for the collection of high-quality spectra with distinct spectral features that were very consistent within each sample.

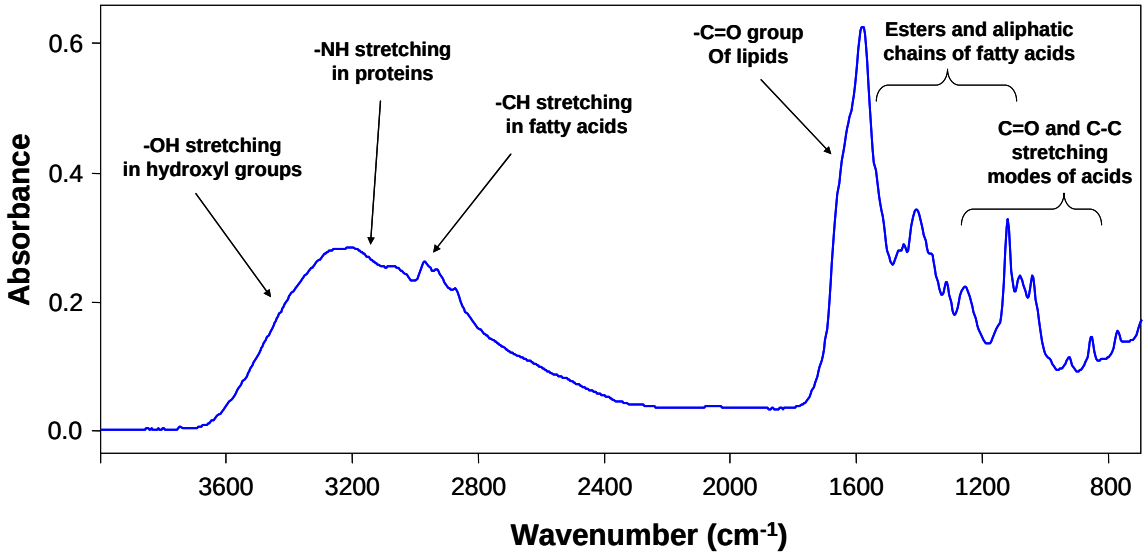

Figure 1. Typical FT-IR spectra of Cheddar cheese extract. Exactly 10 µL of the extract was dried on zinc selenide crystal and scanned in the mid-infrared region (4000 to 700 cm^{-1}). Important functional groups and their region of absorbance are highlighted.

The FT-IR spectra reflect the total chemical composition of the cheese extract, with absorbance bands due to acids, esters, alcohols and peptides. The peak intensities vary with the overall concentration of the chemical functional groups in the sample. Typical FT-IR spectrum of Cheddar cheese extract is shown in **Figure 1**. The region from 4000 to 3100 cm^{-1} consists of

absorbance from O-H and N-H stretching vibrations of hydroxyl groups and Amide A of proteins, respectively. The C-H stretching vibrations of $–CH_3$ and $>CH_2$ functional groups of long chain fatty acids appear between 3100 and 2800 cm^{-1}. The spectral range 1800 to 900 cm^{-1} contains signal from proteins, carbonyl groups of fatty acids, hydroxyl groups, carboxylic acid groups and fatty acid esters (typically short chain). Visual comparison of the raw spectra showed numerous differences between cheeses in the spectral regions 1500-900 cm^{-1}. The raw FT-IR spectra were normalized, standardized and transformed into their second derivatives prior to multivariate analyses to remove the baseline shifts, improve the peak resolution, and reduce the variability between replicates (Kansiz et al., 1999).

The transformed spectra were correlated with the age of the cheese, composition (fat, salt, and moisture), and pH determined by reference methods to develop PLSR models (**Figure 2**). All the five models exhibited excellent correlation with coefficient of correlation (r) values greater than 0.95. The age of the cheese could be predicted with a standard error of cross-validation (SECV) value of 1.77 days (**Figure 2A**). The SECV is an estimate of the error expected when independent samples are predicted using the model. Predicting the age of a cheese can provide valuable information for monitoring cheese ripening to achieve an acceptable endpoint. Similarly, the models for predicting fat content, salt, moisture, and pH of the cheese samples exhibited very low SECV values of 0.21% (**Figure 2B**), 0.19% (**Figure 2C**), 0.15% (**Figure 2D**), and 0.01 (**Figure 2E**), respectively. Preliminary results with amino acid and organic acid estimation also provided similar results (data not shown). Currently, determination of composition and pH require the use of multiple techniques and several organic chemicals. Furthermore, these methods are complicated and expensive. The results emphasize the capability of FT-IR spectroscopy to rapidly and reliably predict cheese characteristics.

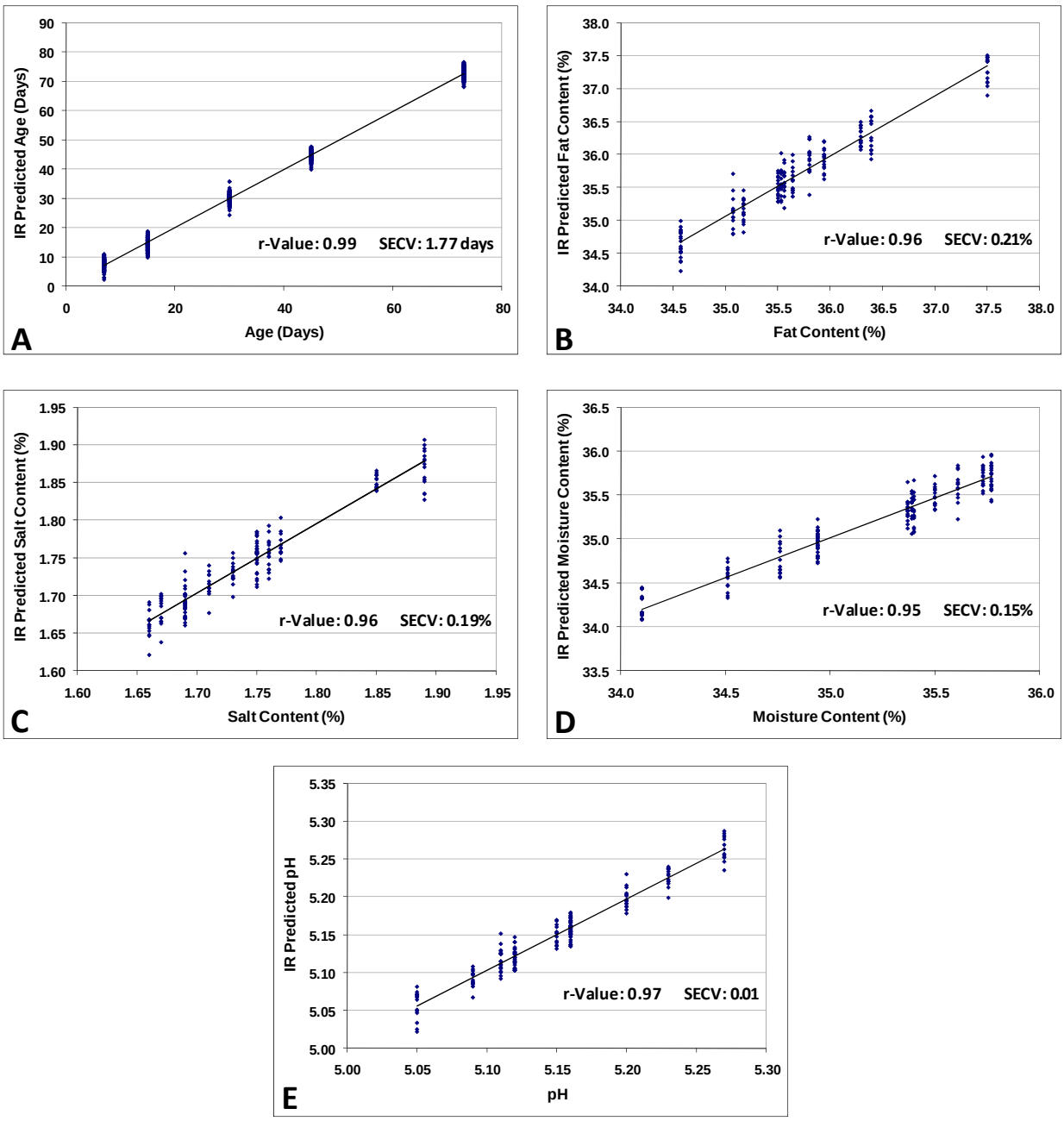

Figure 2. Partial least squares regression models for prediction of A) Age (*n=941*), B) Fat (*n=182*), C) Salt (*n=182*), D) Moisture (*n=182*), and E) pH (*n=182*) of cheese samples. The spectra were transformed into their second-derivative, mean-centered and normalized prior to multivariate analyses.

Three dimensional classification model based on SIMCA was developed to discriminate cheeses based on their flavor quality. The SIMCA model was developed by computing a small number of orthogonal variables (the principal components or PCs) that explained as much of the variation as possible between the samples, while preserving the relevant information and eliminating random noise (Mark, 2001). SIMCA classification plot, a projection of the original data onto the principal components, allowed the visualization of well-separated clustering among the samples, whose orientation in 3D space correlated with their flavor quality. SIMCA also provides a 95% probability cloud, which means that there is 95% probability that the samples within that cloud belong to the same flavor category. For clarity the SIMCA classification plot of 8 of the 12 fully ripe (73 days old) samples is shown in **Figure 3**.

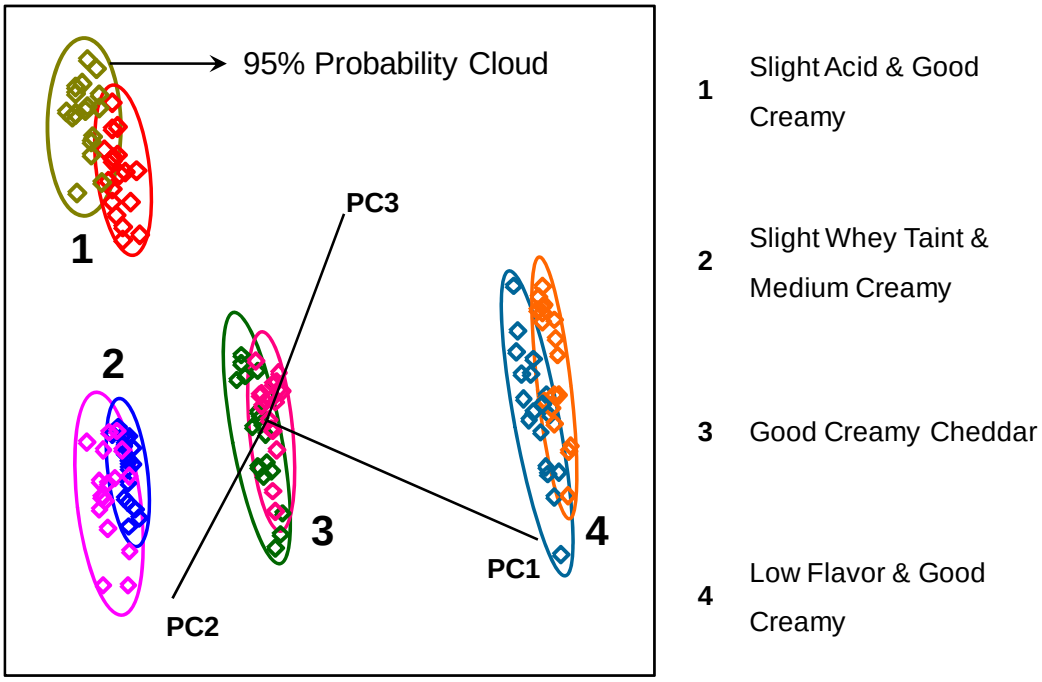

Figure 3. Soft independent modeling of class analogy classification plot for discrimination of cheese samples based on flavor quality. The samples were projected against the first three principal components (PC) that explained the largest amount of variance among the samples.

All the eight samples formed tight clusters and the location of the clusters in 3D space correlated well with their flavor quality. The good samples clustered together (cluster 3) and away from the samples with defects. The distance between the clusters in a SIMCA plot is represented by the interclass distance (ICD). Greater the distance between two clusters the greater is the difference in composition of samples belonging to those clusters. As a rule of thumb, a distance of over 3 indicates that the samples are well separated (Kyalheim and Karstand, 1992). Samples that had the same flavor quality had ICD of less than 3 within themselves and an ICD of greater than 3 when compared to samples with a different flavor quality. This clearly indicates the potential of using FT-IR spectroscopy with multivariate analysis to predict the flavor quality of cheese.

The spectral wavenumbers and the associated functional groups that were responsible for the classification of the cheeses in SIMCA plot can be identified using the discriminating power plot. In the discriminating power plot each wavenumber in the spectral range is plotted against its importance in discriminating the samples that are in the model. The higher the value of discriminating power, the greater is the influence of that wavenumber in classifying the samples. The spectral regions and the associated functional groups/compounds responsible for the differentiation of the Cheddar cheese samples are highlighted in **Figure 4**. The spectral range 1800 – 900 cm^{-1} was found to be important in the analysis of cheese flavor by FT-IR. This region consists of signals from C-O and C=O (~1175 cm^{-1}), C-H bending (~1450 cm^{-1}), esters (1750-1700 cm^{-1}) and C-O stretching (~1240 and 1170 to 1115 cm^{-1}) (Rodriguez-saona et al., 2006). In the case of Cheddar cheese extract compounds containing these functional groups include the organic acids, alcohols, short chain fatty acids and their esters, amino acids and small water soluble peptides.

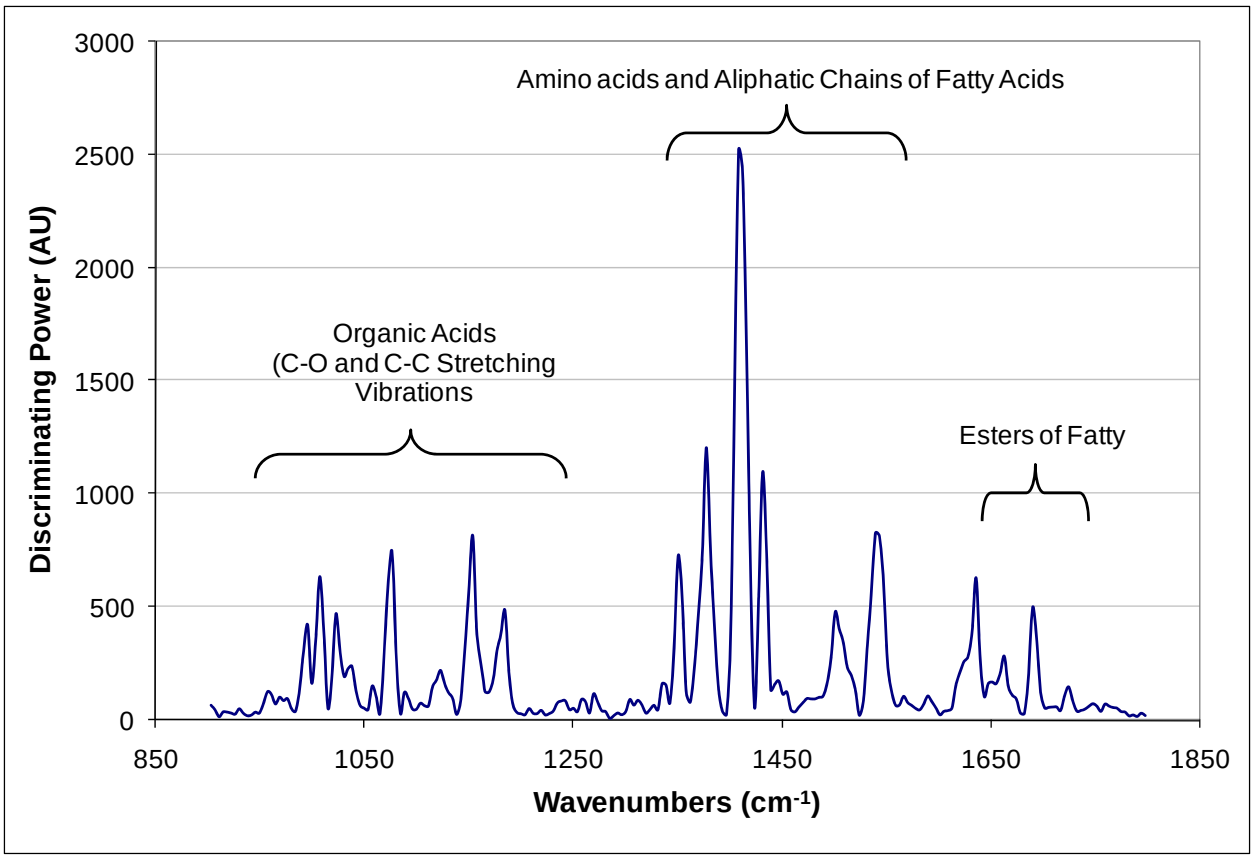

Figure 4. Discriminating power plot for classification of Cheddar cheese samples. The regions of the FT-IR spectra that contribute to the discrimination of the cheese samples based on their flavor are highlighted. Higher the discriminating power at a particular wavenumber the greater is the difference between the samples in the chemical groups associated with that wavenumber.

Apart from determination of age, composition and flavor quality of the cheeses, this technique also enabled monitoring some of the biochemical reactions that took place during cheese ripening. For example, the changes that occurred during various stages of ripening in a sample with a final flavor quality of "Good Creamy Cheddar" are shown in **Figure 5.** Between day 7 and day 15, minor changes occurred in fatty acid and amino acid composition, which could potentially represent initial stages of fat and protein breakdown. The period from day 15 to day 30 exhibited the greatest amount of changes possibly due to heightened protein and fat

breakdown (1550 – 1300 cm^{-1} and 1710 cm^{-1}). The third stage (day 30-day 45) shows the appearance of breakdown products, especially organic acids (1200-900 cm^{-1}). The final stage (day 45-day 73) of Cheddar cheese ripening showed relatively less intense but numerous changes in the regions that correspond to amino acids, short chain fatty acids and organic acids, which could signify formation of flavor related minor compounds.

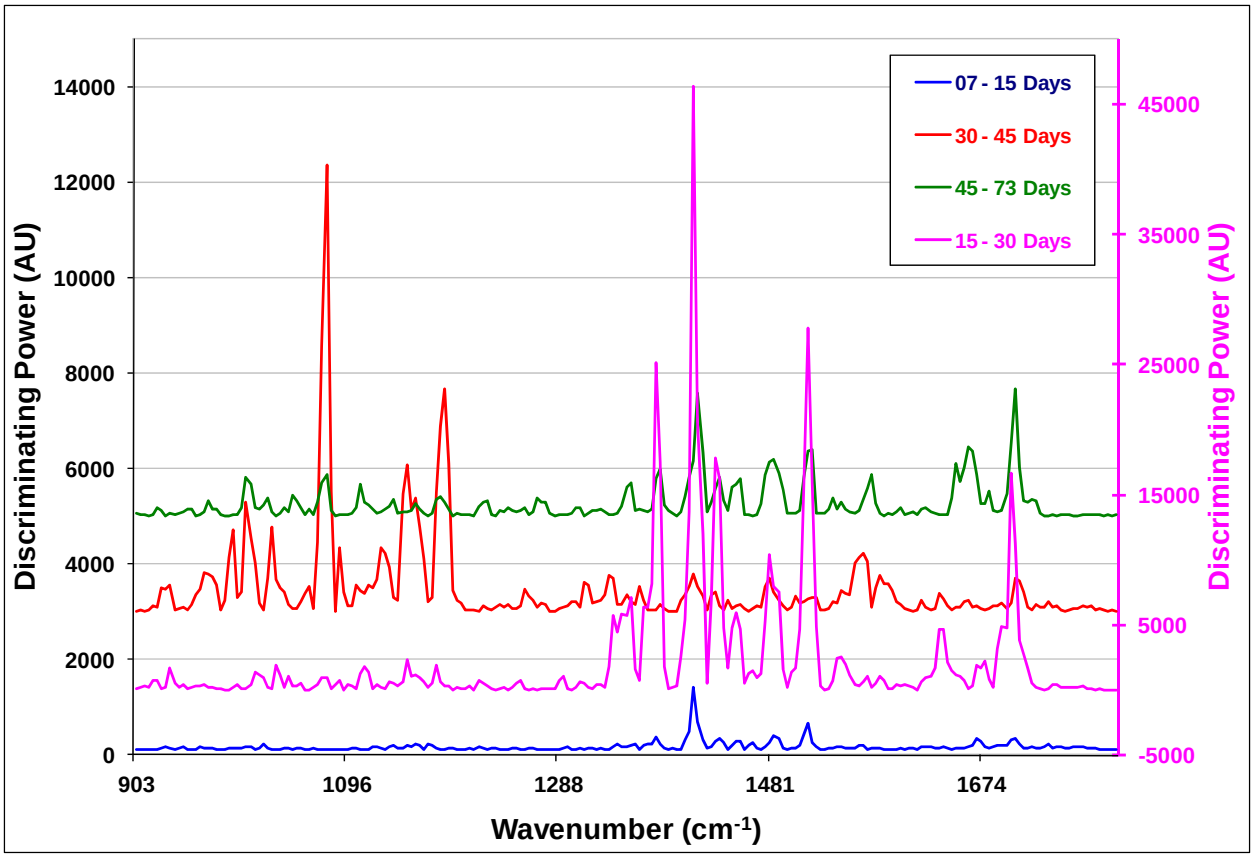

Figure 5. Biochemical changes in Cheddar cheese that occurred between days 7 and 15 (blue), 15 and 30 (purple), 30 and 45 (red), and 45 and 73 (green) of ripening. Higher the discriminating power the greater is the change. The period from day 15 to day 30 (secondary axis) exhibited greatest change during the ripening process.

Thus, the described FT-IR technique could provide valuable information on flavor related biochemical changes during ripening. Such information could be of help in understanding flavor

formation during cheese ripening as well as monitoring and controlling cheese ripening process to achieve desired flavor formation.

Conclusions

A rapid, simple, and reliable FT-IR technique was developed for simultaneous analysis of Cheddar cheese composition and flavor quality. The age, fat, salt, moisture, pH and flavor quality of the cheese could be predicted in less than 20 min. Furthermore, the technique also provided insights into the changes occurring during cheese ripening process. Preliminary experiments with Swiss cheese provided well defined spectra and very good classification. Therefore, this technique has the potential of being applied to not only Cheddar cheese but also other types of cheese. The developed technique shows great promise and can save time and money for the cheese industry. It will enable better quality control and rapid monitoring of ripening process to achieve cheese of desired flavor quality. Additionally, this method will also initiate and accelerate further studies on cheese ripening process using FT-IR spectroscopy.

Works Cited

Adda, J., Gripon, J.C., and L. Vassal. 1982. The chemistry of flavor and texture generation in cheese. Food Chem. 9:115-129.

Akalin, A.S., Gonc, S., and Y. Akba. 2002. Variation in organic acids content during ripening of pickled white cheese. J. Dairy Sci. 85:1670-1676.

Alli, I., Okoniewska, M., Gibbs, B.F., and Y. Konishi. 1998. Identification of peptides in Cheddar cheese by electrospray ionization mass spectrometry. Int. Dairy J. 8:643-649.

Bachmann, HP., Butikofer, U., and J. Meyer. 1999. Prediction of flavor and texture development in Swiss-type cheeses. Lebensm.-Wiss. U.-Technol. 32:284-289.

Chen, M., and J. Irudayaraj. 1998. Sampling technique for cheese analysis by FTIR spectroscopy. J. Food Sci. 63:96-99.

Chen, M., Irudayaraj, J., and D.J. McMahon. 1998. Examination of full fat and reduced fat Cheddar cheese during ripening by Fourier transform infrared spectroscopy. J. Dairy Sci. 81:2791-2797.

Farkye, N.Y., and P.F. Fox. 1990. Objective indices of cheese ripening. Trends Food Sci. Technol. 1:37-40.

Kansiz, M., Heraud, P., Wood, B., Burden, F., Beardall, J., and D. McNaughton. 1999. Fourier transform infrared microspectroscopy and chemometrics as a tool for the discrimination of cyanobacterial strains. Phytochemistry. 52, 407-417.

Kosikowski, F.V., and G. Mocquot. 1958. Advances in cheese technology. FAO Agric. Stud. Nr. 38, Rome, Italy: Food and Agriculture Organization (FAO). 15 p.

Kvalheim, O.M., and T.V. Karstand. 1992. SIMCA – classification by means of disjoint cross validated principal components models. In: Brereton, R.G. (Eds.), Multivariate patten recognition in chemometrics: illustrated by case studies. Elsevier, Amsterdam, Netherlands, pp 209-248.

Lues, J.F.R., and A.C.M. Bekker. 2002. Mathematical expression for organic acids in early ripening of a Cheddar cheese. J. Food Compos. Anal. 15:11-17.

Mark, H. 2001. Multilinear regression and principal component analysis. In: Burns, D.A., Ciurczak, E.W. (Eds.), Handbook of Near-Infrared Analysis. Taylor and Francis, New York, pp. 129-184.

McQueen, D.H., Wilson, R., Kinnunen, A., and E.P. Jensen. 1995. Comparison of two infrared spectroscopic methods for cheese analysis. Talanta. 42:2007-2015.

McSweeney, P.L.H., and M.J. Sousa. 2000. Biochemical pathways for the production of flavour compounds in cheeses during ripening: A review. Lait. 80, 293-324.

National Agricultural Statistics Service, United States Department of Agriculture, http://www.nass.usda.gov/, Accessed on March 31, 2007.

Partidario, A.M., Barbosa, M., and L.V. Boas. 1998. Free fatty acids, triglycerides and volatile compounds in Serra da Estrela cheese-changes throughout ripening. Int. Dairy J. 8:873-881.

Rodriguez-saona, L.E., Koca, N., Harper, W.J., and V.B. Alvarez. 2006. Rapid determination of Swiss cheese composistion by Fourier transform infrared/attenuated total reflectance spectroscopy. J. Dairy Sci. 89:1407-1412.

Seitz, E.W. 1990. Microbial- and enzyme-induced flavor in dairy foods. J. Dairy Sci. 73:3663-3691.

Singh, TK., Drake, M.A., and K.R. Cadwallader. 2003. Flavor of Cheddar cheese: A chemical and sensory perspective. Comp. Rev. Food Sci. Food Saf. 2: 166-189.

Thierry, A., Maillard, M.B., and J.L. Le Quere. 1999. Dynamic headspace analysis of Emmental aqueous phase as a method to quantify changes in volatile flavor compounds during ripening. Int. Dairy. J. 9:453-463.

Urbach, G. 1993. Relations between cheese flavor and chemical composition. Int. Dairy J. 3:389-422.

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