The Effect of Natural Cheddar Cheese Ripening on the Functional and Textural Properties of the Processed Cheese Manufactured Therefrom

C.A. BRICKLEY, M.A.E. AUTY, P. PIRAINO, AND P.L.H. MCSWEENEY

ABSTRACT: Cheddar cheese ripened at 8 °C was sampled at 7, 14, 28, 56, 112, and 168 d and subsequently used for the manufacture of processed cheese. The cheddar cheese samples were analyzed throughout ripening for proteolysis while the textural and rheological properties of the processed cheeses (PCs) were studied. The rate of proteolysis was the greatest in the first 28 d of cheddar cheese ripening but began to slow down as ripening progressed from 28 to 168 d. A similar trend was observed in changes to the texture of the PC samples, with the greatest decrease in hardness and increase in flowability being in the first 28 d of ripening. Confocal scanning laser microscopy showed that the degree of emulsification in the PC samples increased as the maturity of the cheddar cheese ingredient increased from 7 to 168 d. This increased emulsification resulted in a reduction in the rate of softening in the PC in samples manufactured from cheddar cheese bases at later ripening times. Multivariate data analysis was performed to summarize the relationships between proteolysis in the cheddar cheese bases and textural properties of the PC made therefrom. The proportion of α_{s1}-casein (CN) in the cheddar cheese base was strongly correlated with hardness, adhesiveness, fracturability, springiness, and storage modulus values for the corresponding PC. Degradation of α_{s1}-CN was the proteolytic event with the strongest correlation to the softening of PC samples, particularly those manufactured from cheddar cheese in the first 28 d of ripening.

Keywords: Cheddar cheese, functionality, processed cheese, proteolysis, texture

Introduction

Processed cheese (PC) is produced by comminuting and melting one or more natural cheeses and optional ingredients (dairy or nondairy) into a smooth, homogenous molten blend using heat, mechanical shear, and emulsifying salts (Caric and Kalab 1993). Production of PC has been increasing steadily, with production being estimated at approximately 1.5 to 1.8 million tons per year, equal to approximately 10% to 12% of natural cheese production (Guinee 2002). Processed cheese is popular as it has many advantages over natural cheese (Fox and others 2000), including better keeping qualities, good diversity of type and flavor intensity, the possibility to utilize natural cheeses with cosmetic defects, and also suitability for use both in the home and in the fast-food industry. Three main groups of PCs are recognized: PC blocks, PC foods, and PC spreads, and these are differentiated by composition, water content, and consistency (Caric and Kalab 1993).

The textural and rheological properties of PC are the major determinants of its quality (Guinee 2002) and a number of factors have been reported to influence these properties, such as composition, pH, type, and level of emulsifying salts and maturity of the natural cheese, in addition to a number of processing parameters, including time, temperature, and shear rate (Guinee and others 2004). Natural cheese is the main ingredient of PC and so the properties of the natural cheese (type, pH, texture, flavor, consis-

tency, and maturity) are extremely important for the final quality of PC (Caric and Kalab 1993). Three major biochemical events (proteolysis, lipolysis, and the metabolism of lactose, lactate, and citrate) take place during cheese ripening which, together with the solubilization of colloidal calcium phosphate (CCP) (Lucey and others 2003), cause the development of flavor and textural changes during ripening of natural cheese.

Proteolysis is inversely related to the levels of intact casein in cheese (Fenelon and Guinee 2000). The levels of intact casein in the cheese (Berger and others 1998) as well as its pH and calcium to casein ratio influence the extent of casein hydration during the manufacture of PC. The levels of intact casein in the cheese system will also, in turn, influence the degree of emulsification, the degree of casein aggregation, and the elasticity of the final PC (Guinee and others 2004). At commercial level, block PC with good sliceability and elasticity is manufactured using young natural cheese with 75% to 90% intact casein remaining while PC spreads are manufactured using mature natural cheese containing 60% to 75% intact casein (Fox and others 1996). Processed cheese manufacturers carefully select the ratio of young to mature natural cheese in the blend in order to take full advantage of the positive attributes of both. However, excessive proteolysis in natural cheese has been associated with textural defects in PC manufactured therefrom, including overshortness, faulty body, and graininess (Lazaridis and others 1981). Templeton and Sommer (1930) reported that PC made from mature natural cheese was softer compared to PC made from unripened cheese. Olson and others (1958) and Vakaleris and others (1962) suggested that the extent of proteolysis in the natural cheese ingredient may influence the body of PC spreads. However, these authors recognized that a clear explanation could not be offered about the differences in the body characteristics of the PC

spreads due to lack of studies conducted on cheese proteins and the noncheese constituents.

Although it has been suggested that the extent of proteolysis in the natural cheese ingredient has an effect on the properties of the PC manufactured therefrom, a thorough study of this topic has not yet been conducted. Therefore the objectives of this study were to investigate the extent of proteolytic change that takes place in cheddar cheese used for the manufacture of PC and how these changes may affect the texture of the PC manufactured therefrom.

Materials and Methods

Cheese manufacture

Three 20-kg blocks of 1-d-old cheddar cheese were supplied by a local commercial cheese manufacturer and ripened at 8 °C for 168 d. Throughout this ripening period, the cheese blocks were sampled at 7, 14, 28, 56, 112, and 168 d and subsequently used for the manufacture of PC. At each time point, 1 batch of PC was made from each of the 3 blocks of cheddar cheese. Processed cheese was prepared in a Stephan Vacuum Vertical Mixer (Stephan Machinery Corp., Mundelein, Ill., U.S.A.) by melting a mix of cheddar cheese (90%), butter oil (3%), water (5%), and emulsifying salts (2%) (trisodium citrate, 0.5%, disodium phosphate, 0.75%, and trisodium phosphate, 0.75%; Sigma-Aldrich Chemie Gmbh, Munich, Germany) at 85 °C for 4 min at 1500 rpm. The molten cheese was then hot-filled into rectangular molds, cooled to 4 °C, and held overnight at that temperature. The PC blocks (2 kg) were then removed from the molds, vacuum packed, and stored at 4 °C for 7 d before analysis.

Analysis of composition

Compositional analysis was completed on both the cheddar and processed cheeses at all time points. The pH was measured on a cheese slurry made from 20 g of cheese and 12 g of water as described by the BSI (1976). The moisture contents of the cheeses were determined by an oven drying method (IDF 1982), protein by the macro-Kjeldahl procedure (IDF 1964), salt by a titrimetric method using potentiometric endpoint determination (Fox 1963), and fat by the Gerber method (IIRS 1955). In order to determine total calcium content, cheese (5 g) was slurried in 10 mL of 0.1 M trisodium citrate (pH 8). This mixture was then heated to 37 °C, mixed with an equal volume of 24% (w/v) trichloroacetic acid, and filtered through Whatman No. 113 paper. An aliquot (5 mL) of the filtrate was mixed with 5 mL 10% (w/v) lanthanum chloride and the mixture was diluted to 100 mL with deionized water (Milli-Q Reagent Water System; Millipore Corp., Bedford, Mass., U.S.A.). The sample was analyzed using an atomic absorption spectrophotometer (Varian Associates Inc., Walnut Creek, Calif., U.S.A.). The concentration of calcium in the sample was determined by comparing the emission of the sample against a standard curve prepared using standard solutions containing 0 to 10 ppm calcium. Each analysis was performed in triplicate.

Assessment of proteolysis

The pH 4.6 soluble and insoluble fractions of the cheddar cheeses were prepared by the method of Kuchroo and Fox (1982), including the slight modifications made by Sousa and McSweeney (2001). The protein content of the pH 4.6 soluble fraction (pH 4.6 SN/TN) was determined by the macro-Kjeldahl method (IDF 1964). Ethanol (70%) soluble and insoluble subfractions of the pH 4.6 soluble extracts were prepared according to the method described by Sousa and McSweeney (2001). Urea-polyacrylamide gel electrophoresis (urea-PAGE; 12.5% T, 4% C, pH 8.9) of the

pH 4.6 insoluble fraction of each of the cheddar cheeses at each ripening time point was performed using the procedure of Andrews (1983) with modifications as described by Shalabi and Fox (1987). Gels were stained directly by the method of Blaksely and Boezi (1977). Reverse-phase high-performance liquid chromatography (RP-HPLC) peptide profiles of the ethanol-soluble fractions of each of the cheddar cheeses were determined according to the method described by Sousa and McSweeney (2001). Total free amino acids (FAA) were determined by the trinitrobenzenesulphonic acid (TNBS) assay as described by Polychroniadou (1988).

Processed cheese functionality

Flowability was described as the percent increase in diameter of a cheese disc on heating in a microwave oven (Dimension 4 Microwave oven, Panasonic, Berkshire, U.K.) at full power as described by Bogenreif and Olson (1995).

Rheological and textural analysis

Texture profile analysis (TPA) of the PC was performed using a TA-XT2i Texture Analyser (Stable Micro System Ltd., Godalming, Surrey, U.K.). Processed cheese samples were cut into cylinders (20-mm dia, 10-mm height) and stored overnight at 8 °C. During analysis, the samples underwent a double compression to 25% of their original height at a test speed of 1 mm/s. The probe used was a stainless steel cylinder P50 (50 mm in diameter). The TPA parameters recorded were hardness, fracturability, adhesiveness, and springiness (Bourne 1978). The values found were the mean of 5 replicates.

The samples were also analyzed by low amplitude strain oscillation on a dynamic low amplitude oscillatory rheometer (Carrimed CSL^2_{500}; TA Instruments Inc., New Castle, Del., U.S.A.) as described by Guinee and others (1999). Cheese discs (40-mm dia, 2 mm thick) were held at room temperature for 15 min before being placed between 2 parallel serrated plates of the rheometer cell. The samples were first equilibrated at 20 °C for 3 min and then subjected to a low-amplitude shear strain of 0.005 at an angular frequency of 1 Hz. Temperature was increased from 20 to 82 °C at a rate of 3 °C/min (Guinee and others 2002). The parameters recorded were storage modulus (G′) and tan delta (TD) and these were measured every 20 s during heating. Each sample was analyzed in triplicate.

Confocal scanning laser microscopy of unheated processed cheeses

Slices of cheese, measuring 5 × 5 × 2 mm, were cut from a freshly cut block (at 4 °C) using a razor blade. Slices were taken in 3 perpendicular planes of the PC. To label protein and fat phases simultaneously, 100 μL of a dual-labeling fluorescent staining mixture was applied to the cut horizontal surface of the PC and a coverslip placed on top. The fluorescent staining mixture was prepared as follows: 100 μL of a 1.0 g/L aqueous solution of Fast Green FCF was added to 100 mL of polyethylene glycol (molecular weight 400 Da) containing 0.2 g/L Nile Red. Fast Green FCF labels protein when excited at 633 nm and Nile Red labels fat when excited at 488 nm (Auty and others 2001). A Zeiss LSM 310 confocal microscope (Carl Zeiss Ltd., Welwyn Garden City, Hertfordshire, U.K.) was used for acquiring images of the PC samples manufactured from cheddar cheeses ripened to 7, 28, or 168 d. The confocal pinhole diameter was set to give an *x–y* resolution approximately 0.2 μm and an axial resolution of approximately 1.0 μm. Color images (24 bit), 512 × 512 pixels in size, were acquired using zoom factors of either 1.0, 2.0, or 3.0 to give final pixel resolutions of 0.2, 0.1, or 0.05 μm/pixel, respectively.

Table 1—Composition, pH, levels of total free amino acids (expressed as mg leucine/g cheese), and levels of pH 4.6-soluble nitrogen expressed as a percentage of total nitrogen (pH 4.6 SN/TN) for the cheddar cheeses for 7, 14, 28, 56, 112, or 168 d.

Cheese age (days)	Moisture (%)	Fat (%)	Protein (%)	Salt (%)	S/M[A] (%)	pH	mg Leucine/g cheese	pH 4.6 SN/TN
7	37.0 ± 0.6[a]	30 ± 0.01[a]	26.4 ± 1.1[a]	1.99 ± 0.06[a]	5.38 ± 0.14[a]	5.27 ± 0.03[a]	12 59 ± 0.83[a]	5.19 ± 0.04[a]
14	37.0 ± 0.8[a]	29 ± 0.04[a]	26.7 ± 0.4[a]	1.99 ± 0.13[a]	5.39 ± 0.37[a]	5.18 ± 0.09[b]	14.57 ± 0.99[a]	5.69 ± 0.12[a]
28	37.5 ± 0.8[a]	29 ± 0.08[a]	26.8 ± 0.3[a]	2.00 ± 0.03[a]	5.48 ± 0.32[a]	5.30 ± 0.04[a]	18.99 ± 0.60[b]	7.53 ± 0.45[b]
56	37.5 ± 0.5[a]	28 ± 0.10[a]	26.7 ± 0.4[a]	1.99 ± 0.10[a]	5.33 ± 0.25[a]	5.31 ± 0.03[a]	33.61 ± 2.64[c]	11.32 ± 0.32[c]
112	37.8 ± 0.8[a]	30 ± 0.08[a]	26.4 ± 0.3[a]	2.00 ± 0.08[a]	5.47 ± 0.15[a]	5.21 ± 0.05[b]	49.88 ± 0.30[d]	15.57 ± 0.10[d]
168	37.8 ± 0.9[a]	30 ± 0.60[a]	26.6 ± 0.2[a]	1.98 ± 0.06[a]	5.22 ± 0.02[a]	5.18 ± 0.03[b]	55.80 ± 1.29[e]	20.90 ± 0.87[e]

[A]S/M = salt in moisture.
Superscripts with different letters in same column are significantly different ($P < 0.05$). The results are expressed as mean ± standard deviation of triplicate trials.

Table 2—Composition and pH of processed cheeses made using cheddar cheeses ripened for 7, 14, 28, 56, 112, or 168 d.

Ripening time of cheddar cheese base (days)	Moisture (%)	Calcium (mg/g cheese)	Fat (%)	Protein (%)	NaCl (%)	S/M[A] (%)	pH
7	38.2 ± 0.6[a]	38.1 ± 1.0[a]	29 ± 0.01[a]	24.5 ± 0.4[a]	1.93 ± 0.05[a]	5.03 ± 0.06[a]	5.92 ± 0.03[a]
14	38.8 ± 0.6[a]	39.6 ± 0.7[a]	30 ± 0.01[a]	24.5 ± 0.4[a]	1.88 ± 0.08[a]	4.86 ± 0.10[a]	5.96 ± 0.04[a]
28	38.1 ± 0.4[a]	38.4 ± 0.1[a]	30 ± 0.02[a]	24.3 ± 0.3[a]	1.84 ± 0.06[a]	4.82 ± 0.11[a]	6.00 ± 0.03[a]
56	38.3 ± 0.4[a]	38.3 ± 0.1[a]	30 ± 0.02[a]	23.8 ± 0.6[a]	1.89 ± 0.08[a]	4.93 ± 0.17[a]	5.99 ± 0.03[a]
112	37.7 ± 0.5[a]	38.9 ± 0.1[a]	30 ± 0.01[a]	24.9 ± 0.1[a]	1.88 ± 0.03[a]	5.00 ± 0.04[a]	5.98 ± 0.04[a]
168	38.1 ± 0.7[a]	39.9 ± 1.2[a]	30 ± 0.01[a]	24.1 ± 0.4[a]	1.91 ± 0.02[a]	5.01 ± 0.08[a]	5.92 ± 0.01[a]

[A]S/M = salt in moisture.
Superscripts with different letters in same column are significantly different ($P < 0.05$). The results are expressed as mean ± standard deviation of triplicate trials.

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Statistical analysis

The data from RP-HPLC chromatographic analysis of the ethanol-soluble fractions of the cheddar cheeses were analyzed using multivariate statistical techniques. The variables (peak height data) were preprocessed according to the method of Piraino and others (2004). The output from this preprocessing consisted of classes of retention time within which peak heights were accumulated using the distance from center of class as a weight. Principal component analysis was then performed on the data using a covariance matrix (Pripp and others 1999) using the SPSS statistical package (SPSS Inc., Chicago, Ill., U.S.A.).

Relationships between indices of proteolysis of the cheddar cheeses (the X matrix) and the rheological properties of the PC samples (the Y matrix) were assessed using partial least squares (PLS) regression. The analysis was carried out using R (Ihaka and Gentleman 1996). The variables were autoscaled so that each variable column had the same weight, the same prior importance in the analysis. The PLS model was built using the Kernal alogorithm. A number of 3 processed cheeses were considered in the model. The model was cross-validated using 3 random segments.

One-way analysis of variance (ANOVA) of data for the composition, levels of pH 4.6 SN/TN, total free amino acids, and TPA analysis of the cheeses was conducted using SPSS Version 14.0 for Windows XP (SPSS Inc.). Significance was declared at $P < 0.05$.

Results and Discussion

Cheese composition

The gross compositions and pH of the cheddar cheeses and the PC made therefrom are shown in Table 1 and 2. The values for pH, moisture, protein, and salt were in the expected ranges of 5.18% to 5.31%, 36.47% to 37.83%, 24.41% to 26.79% and 1.97% to 2.07% for the cheddar cheeses and 5.88% to 6.01%, 37.70% to 38.84%, 23.83% to 24.02%, and 1.84% to 1.97% for the PC samples, respectively. No differences in composition for both the cheddar cheeses and the PC samples were found during ripening. The PC had higher pH values than those of the cheddar cheeses, which was due to the alkaline

nature of the emulsifying salts used during PC manufacture (pH of 1% solution of TSC = 8.3, DSP = 9.8, TSP = 13.0; Fox and others 2000).

Proteolysis

Urea-PAGE electrophoretograms of the pH 4.6 insoluble fractions of cheddar cheeses aged from 7 to 168 d were performed (results not shown). Degradation of α_{s1}-casein (CN) was more extensive than that of β-CN, which is typical for cheddar cheese. Densitometry was performed on the electrophoretograms to represent numerically the levels of the caseins and their degradation products in the cheddar cheese samples (Figure 1). There was a steady breakdown of α_{s1}-CN over time with the most extensive taking place in the first 28 d of ripening. From 7 to 56 d, α_{s1}-CN (f 24 to 199) was produced (O'Mahony and others 2005) while levels of
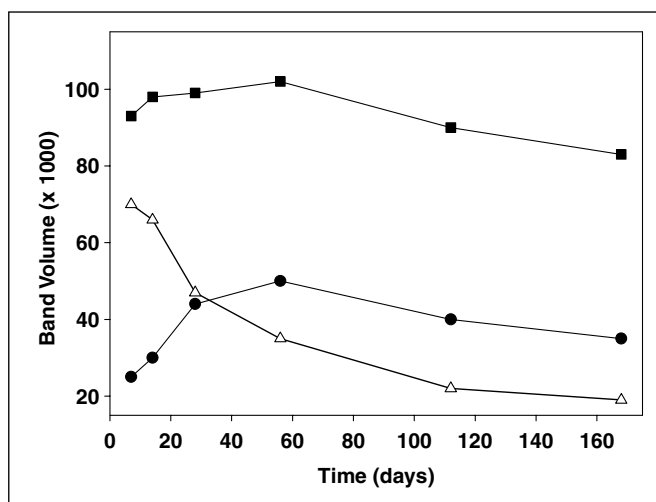

Figure 1—Volume of the bands corresponding to β-casein (■), α_{s1}-casein (△), and the peptide α_{s1}-casein (f 24 to 199) (●) on urea-polyacrylamide gel electrophoretograms of cheddar cheeses ripened for 7, 14, 28, 56, 112, or 168 d. Values are means from 3 replicates.
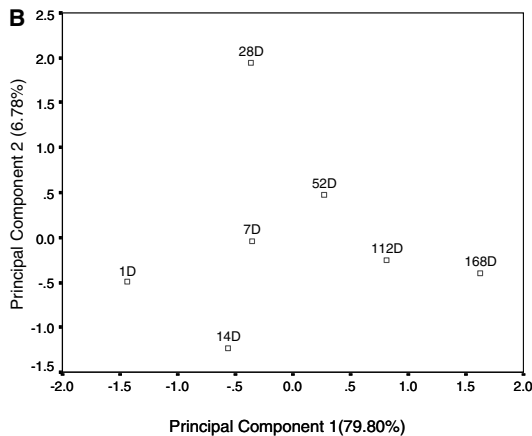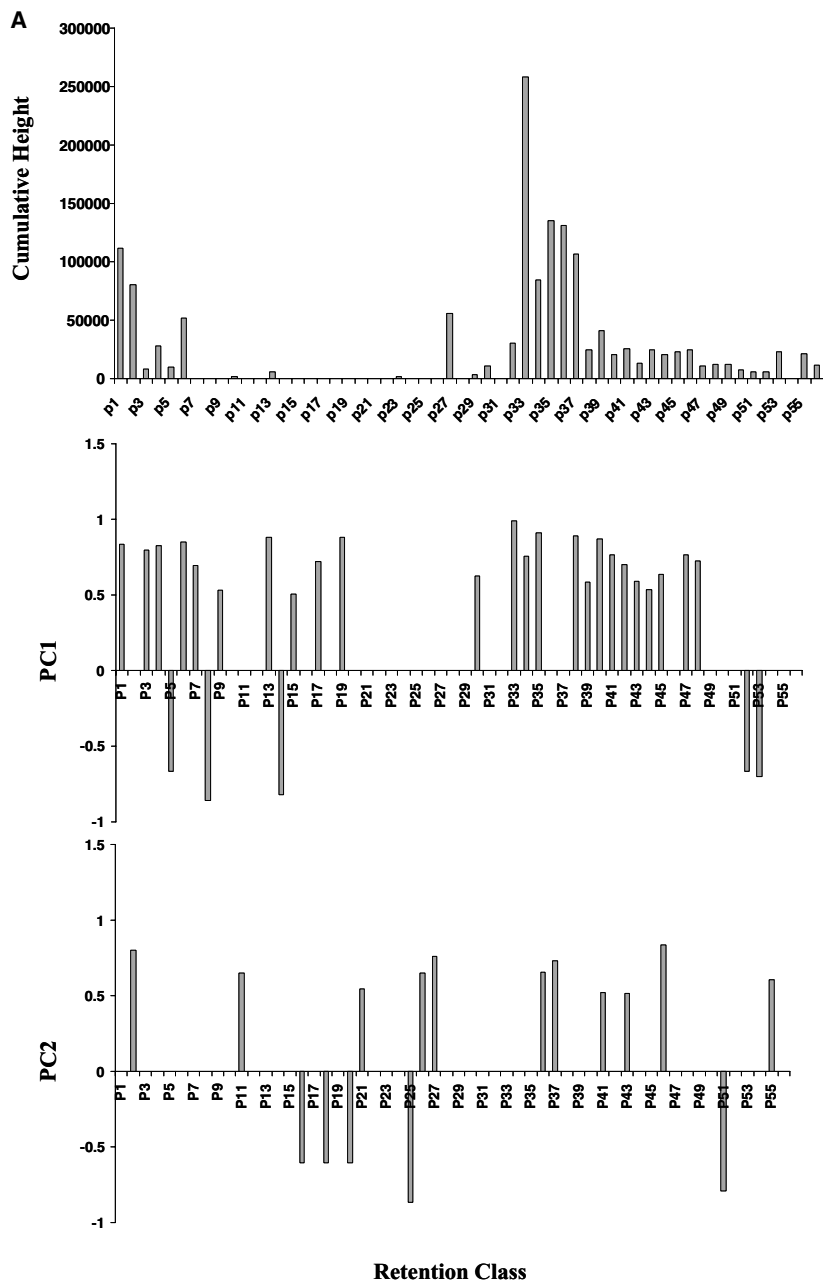
Figure 2 — Factor loadings (A) for principal components 1 and 2 and score plot (B) obtained by principal component analysis of chromatographic data obtained from 70% ethanol-soluble subfractions of pH 4.6 soluble extracts from cheddar cheeses ripened for 7, 14, 28, 56, 112, or 168 d.

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β-CN remained constant. Degradation of both β-CN and α_{s1}-CN (f 24 to 199) occurred from 56 to 168 d.

Levels of pH 4.6 SN/TN, which represent the primary degradation of casein (Fenelon and Guinee 2000), increased in cheddar cheese samples as ripening progressed from 7 to 168 d (Table 1). The rate of primary casein degradation was the greatest from 7 to 56 d, which is in agreement with other reports (Lucey and others 2005; O'Mahony and others 2005). Amino acid production (Table 1) increased steadily as cheddar cheese ripened from 7 to 168 d. O'Mahony and others (2005) also observed a steady increase in amino acid production during ripening for cheddar cheeses containing increasing levels of pepstatin.

Principal component analysis (PCA) was performed on the data from the RP-HPLC chromatograms. Principal components 1 and 2 accounted for 79.80% and 6.78% of the variation, respectively, giving a cumulative variation of 86.58% for the total data from the cheddar samples (Figure 2A). Factor loadings associated with the 1st and 2nd principal components are also shown on Figure 2B. Principal component 1 separated samples on the basis of age and had high positive factor loadings for a group of hydrophobic peptides with intermediate to long retention times. Therefore, although peptides across the entire profile are collectively responsible for differences between cheeses, differences existing between the cheddar samples as ripening progressed can mainly be attributed to changes taking place in this group of hydrophobic peptides.

Microstructure of processed cheese

Confocal scanning laser micrographs of the PC samples manufactured from cheddar cheese ripened for 7, 28, or 168 d are shown in Figure 3. Previous microstructural studies of PC have shown that the structure is an emulsion of round fat globules in a hydrated protein matrix (Tamine and others 1990). As the age of the cheddar cheese base used as an ingredient in PC manufacture increased and consequently levels of intact caseins decreased, there was an increase in the degree of emulsification in the PC samples. This increase in degree of emulsification is represented by the increase in the number and decrease in the mean diameter (from approximately 120 μm for the PC manufactured from the cheddar cheese at 7 d to approximately 2 μm for the PC manufactured with the cheddar cheese at 168 d; Figure 3) of the fat globules in the system (Savello and others 1989). The increased number of emulsified fat globules in the PC in turn contributes to the overall continuity of the PC matrix by acting as fillers (Aguilera and Kessler 1989), resulting in a cheese with a more reinforced structure (Guinee and others 2000).

Texture and rheology

Flowability. As the age of the cheddar cheese ingredient used in the PC manufacture increased, there was an increase in the percent flowability in the PC samples in the range of 12.55% to 20.78% (Table 3). Olson and others (1958) also observed an increase melt in PC spreads as the age of the natural cheese ingredient increased. The majority of this increase took place in the PC manufactured from cheddar cheese ripened from 7 to 56 d. This is also the time period during which degradation of α_{s1}-CN primarily took place (Figure 1). Along with the solubilization of CCP, α_{s1}-CN breakdown in cheese plays a role in the initial softening of cheese during early ripening (Creamer and Olson 1982; O'Mahony and others 2005). Tamime and others (1990) reported that reduced firmness in the PC
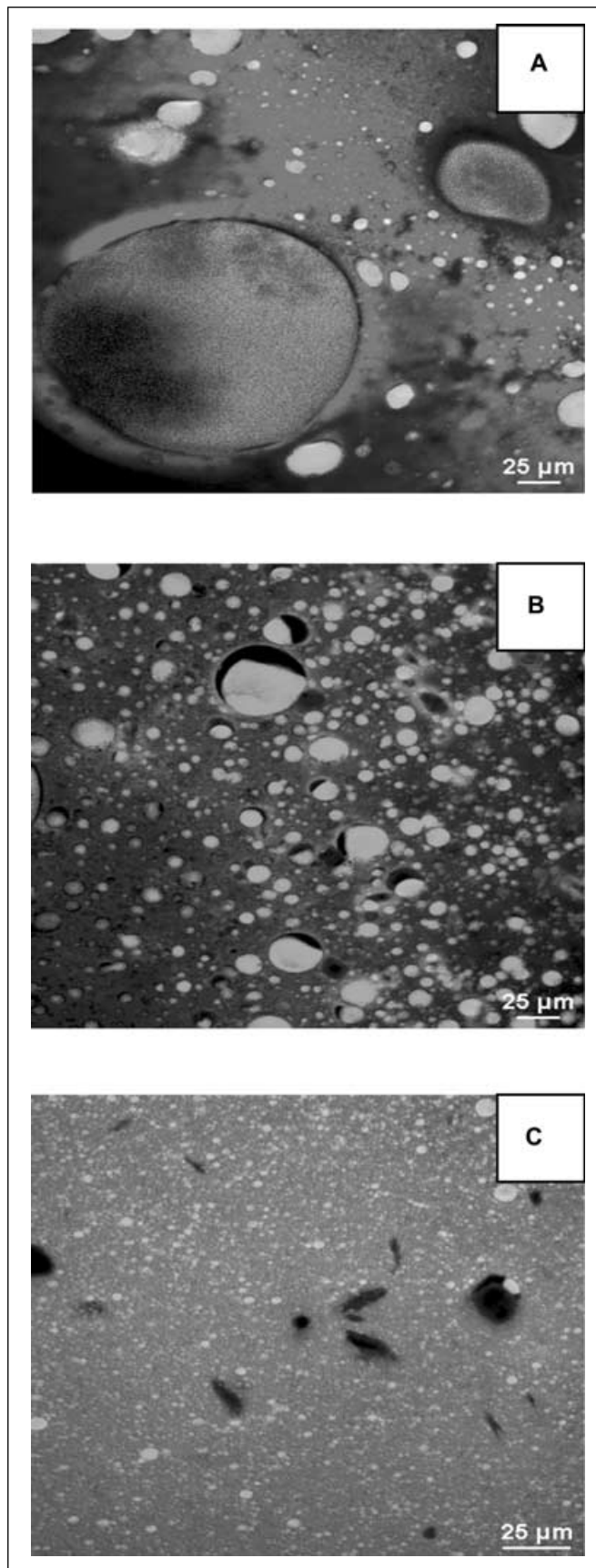

Figure 3 – Confocal scanning laser micrographs of PC samples manufactured from cheddar cheese ripened for 7 d (A), 28 d (B), or 168 d (C). Micrographs show fat globules as approximately spherical bodies on the protein matrix.

Table 3—Hardness, fracturability, adhesiveness, and springiness values as determined using texture profile analysis and percent flowability values for processed cheese samples manufactured using cheddar cheese ripened for 7, 14, 28, 56, 112, or 168 d.

Ripening time of cheddar cheese base (days)	Hardness (N)	Fracturability (N)	Adhesiveness (N/s)	Springiness	Fracture stress (N/mm²)	Flowability (%)
7	171 ± 2.1[a]	194 ± 3.8[a]	778 ± 9.8[a]	1.26 ± 0.05[a]	0.63 ± 0.01[a]	12.6 ± 1.1[a]
14	149 ± 2.4[b]	164 ± 1.6[b]	730 ± 7.1[b]	1.16 ± 0.03[b]	0.48 ± 0.03[b]	14.8 ± 0.6[b]
28	142 ± 2.0[c]	159 ± 0.7[c]	696 ± 5.7[c]	1.11 ± 0.03[c]	0.43 ± 0.02[c]	17.6 ± 0.9[c]
56	125 ± 1.3[d]	153 ± 2.7[d]	681 ± 3.3[d]	0.95 ± 0.03[d]	0.42 ± 0.02[cd]	19.7 ± 0.7[d]
112	120 ± 1.2[e]	142 ± 2.7[e]	655 ± 3.6[e]	0.89 ± 0.02[e]	0.40 ± 0.03[d]	19.8 ± 0.7[e]
168	113 ± 3.0[f]	137 ± 0.8[f]	637 ± 6.5[f]	0.82 ± 0.02[f]	0.40 ± 0.02[d]	20.7 ± 1.1[f]

Superscripts with different letters in same column are significantly different ($P < 0.05$). The results are expressed as mean ± standard deviation of triplicate trials.

samples made from a cheese base produced from skim milk powder may have been due to a reduction in levels of the α_{s1}-caseins in the cheese. The rate of increase in flowability was reduced at later ripening times and this was attributed to the reduced levels of intact caseins in the cheese leading to an increase in the degree of emulsification at these time points. A greater degree of emulsification has previously been reported to result in reduced flowability and increased firmness in the PC samples (Caric and others 1985; Guinee and others 2000).

Texture profile analysis. The values for hardness, adhesiveness, and fracturability in the PC decreased as the maturity of the cheddar cheese base increased (Table 3). Springiness (defined as the height the food recovers between the 1st and 2nd compression; Bourne 1978) also decreased in the PC samples as the cheddar cheese matured. Cheese is a viscoelastic material, meaning that it displays both elastic and viscous properties. Reduced springiness in the samples therefore shows a shift to more viscous properties in the system and therefore a softer texture. Fracture stress (σ_f) also decreased in the PC samples (Table 3), indicating a softening of the PC structure. This was most likely due to a reduction in the levels of intact casein in the cheddar cheese base during ripening which would result in a reduction in the number of casein–casein interactions in the cheese matrix used during processing (Guinee 2002). Again, the greatest reduction in the TPA parameters for the PC samples occurred during the first 56 d of cheddar cheese ripening which is in agreement with the results for percent flowability. Sallami and others (2004) also reported that the majority of the decrease in TPA parameters occurred during the first 30 to 60 d of cheddar cheese ripening which is in agreement with results reported here for the PC. Previous authors (Templeton and Sommer 1930; Vakaleris and others 1962; Garimella Purna and others 2006) have also observed decreased firmness in the PC samples as the age of the natural cheese ingredient was increased which was suggested to be due to the reduced levels of intact casein in the natural cheese. After 56 d ripening of the cheddar cheese, the rate of decrease in the TPA parameters of the PC began to slow. This can again be explained by the greater degree of emulsification in these cheeses; that is, increased contribution of emulsified fat globules to the cheese matrix and the formation of a firmer PC. Also, previous authors have reported evidence showing increased creaming reactions in the PC samples containing mature cheddar cheese (Rayan and others 1980; Kalab and others 1987).

Changes in viscoelasticity during heating. Changes in TD during heating and G' values at 30 °C for the PC samples manufactured from cheddar cheese at 7, 14, 28, 56, 112, or 168 d are shown in Figure 4 and 5. For all PC samples, increasing the temperature resulted in a decrease in G' and an increase in TD. Little increase in TD was evident at temperatures <40 °C; however, at temperatures > 40 °C TD rapidly increased, indicating softening of the cheese and
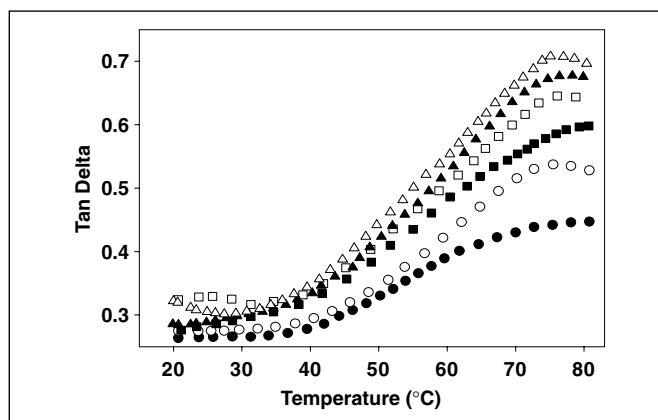

Figure 4—Changes in tan delta for processed cheese samples manufactured from cheddar cheese ripened for 7 (●), 14 (○), 28 (■), 56 (□), 112 (▲), or 168 (△) d on heating from 20 to 82 °C at a rate of 3 °C/min. Values are means from 3 trials.

a change from elastic to viscous properties (Guinee and others 1999). Fat is the only component of cheese that actually melts and since milk fat has completely liquefied by 40 °C (Norris and others 1973), it can be assumed that the increase in TD is due more to the changes that take place in the protein phase of the PC system. Lucey and others (2003) reported that at temperatures > 40 °C, proteins begin to soften, bringing about a melt-like behavior in cheese as a result of the relaxation of specific casein–casein interactions in the system.

Maximum TD increased in the PC samples as the cheddar cheese base matured from 7 to 168 d. Maximum TD is lower at earlier ripening times as there is greater continuity to the protein matrix, that is, high levels of intact casein at these time points resulting in a firmer cheese. The continuity of the protein matrix in the PC manufactured using the cheddar cheese ripened for 7 d can be clearly seen in the confocal scanning laser micrograph shown in Figure 3A. Maximum TD increased from 0.45 to 0.65 during the first 56 d of cheddar cheese base ripening. O'Mahony and others (2006) reported that the higher the maximum TD, the greater the propensity of the cheese to flow when heated and therefore the cheese is softer. The increases in maximum TD values were due to proteolysis in the cheddar cheese base, which resulted in the degradation of the caseins that make up the structural framework of the PC (Fenelon and Guinee 2000). The rate of increase in maximum TD slowed from 56 to 168 d ripening with values in the PC only increasing from 0.65 to 0.71, indicating the presence of more solid-like properties in PC manufactured from cheddar cheeses at later ripening times due to the higher degree of emulsification in these samples and a reduction in the rate of casein degradation.

Figure 5 shows a sharp reduction in G' at 30 °C in the PC manufactured from the cheddar cheese ripened from 7 to 56 d. Storage modulus is a measure of the number and strength of bonds in the cheese system (Lucey and others 2003), and therefore the decrease in G' values represent a reduction in the solid-like properties of the PC. The decrease in G' values at 30 °C occurred during the same time scale as the reduction in the levels of α_{s1}- casein suggesting that changes in the G' values at 30 °C may be related to hydrolysis of α_{s1}- casein. The rate of reduction in G' began to reduce as the maturity of the cheddar cheese used as an ingredient in PC manufacture increased. Taneya and others (1980) reported that high levels of intact casein resulted in extensive protein–protein and protein–fat interactions in PC while reduced levels of intact casein resulted in a decrease in the number of these interactions. Therefore, the PC manufactured in this study from the cheddar ch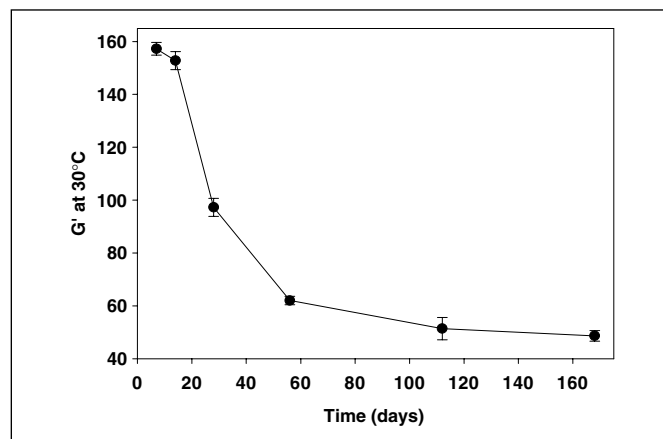eese ripened for short periods had a large number of protein–protein and protein–fat interactions, and consequently higher G' values and lower maximum TD values when compared with the PC manufactured using a mature cheddar cheese (> 56 d of ripening).

Multivariate data analysis

Figure 6 summarizes the results obtained by PLS modeling and gives a picture of the relationships existing between proteolysis in the cheddar cheese samples and rheological and textural properties in the corresponding PC. Five clusters were obtained at a confidence interval of 95%, each corresponding to a time point. The interpretation of the score was achieved by the analysis of the loadings, which are presented as vectors in Figure 6. The rheological and textural properties were modeled with respect to the proteolytic events occurring in the cheddar cheese and are also reported in Figure 6. From the analysis of the scores and loadings the 1st component could be interpreted as "time," and is expressed by the increase in free amino acid concentration and the decrease in α_{s1}-CN. It can be clearly seen from the score plot that from 7 to 56 d α_{s1}-CN (f 24 to 199) was produced extensively but levels of β-CN remained constant. β-CN breakdown occurred from 56 to 168 d. The rheological and textural properties were split over the time axis between those that are high in PC made from young cheese such as hardness and G' and those that increased over time and were subsequently high in PC made from mature cheeses (flowability). From the analysis of the loadings for the response variables it was also observed that the proportion of α_{s1}-CN in the cheddar cheese is strongly correlated with hardness, fracturability, springiness, adhesiveness, and G' values in the PC manufactured therefrom. Flowability was strongly correlated with the production of free amino acids and the development of pH 4.6 SN/TN. The PLS model represented in Figure 6 shows that the degradation of α_{s1}-CN (or equivalently the production of free amino acids and pH 4.6 SN/TN) in the cheddar cheese is the proteolytic event most strongly associated with the softening effect which occurs in the PC samples particularly in the first 28 d of cheddar cheese ripening. The production of α_{s1}-CN (f 24 to 199) and the degradation of β-CN were less

Figure 5 — Changes in the storage modulus (G′) at 30 °C for processed cheese samples manufactured from cheddar cheese ripened for 7, 14, 28, 56, 112, or 168 d. Values are means from 3 trials and error bars show ± standard deviation.
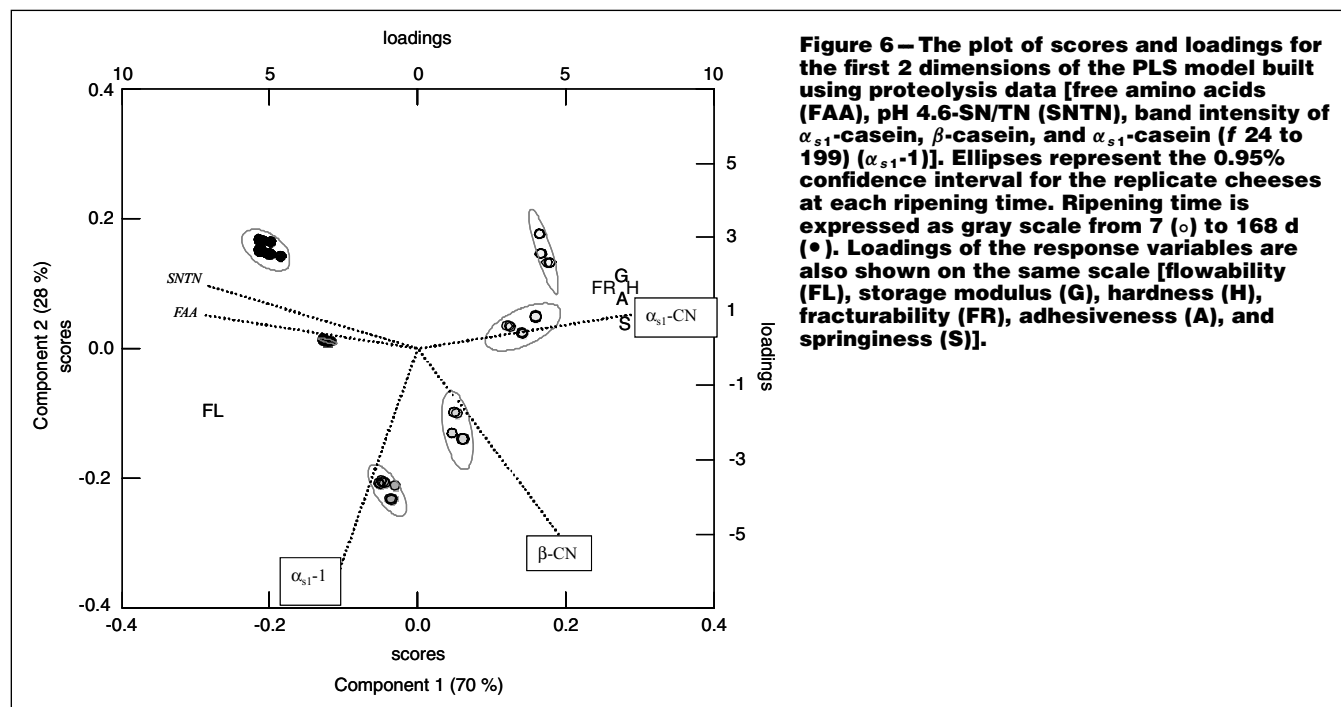

Figure 6 — The plot of scores and loadings for the first 2 dimensions of the PLS model built using proteolysis data [free amino acids (FAA), pH 4.6-SN/TN (SNTN), band intensity of α_{s1}-casein, β-casein, and α_{s1}-casein (f 24 to 199) (α_{s1}-1)]. Ellipses represent the 0.95% confidence interval for the replicate cheeses at each ripening time. Ripening time is expressed as gray scale from 7 (○) to 168 d (●). Loadings of the response variables are also shown on the same scale [flowability (FL), storage modulus (G), hardness (H), fracturability (FR), adhesiveness (A), and springiness (S)].

associated to the functional and textural properties but rather described proteolytic events occurring over time.

Conclusions

The objectives of this study were to investigate the relationship between cheddar cheese ripening, with the emphasis being on proteolytic breakdown, and the resultant textural changes in PC manufactured therefrom. The greatest rate of proteolysis occurs in the first 28 to 56 d of cheddar cheese ripening, which corresponded to a softening of the PC samples manufactured therefrom. Following 56 d ripening of the cheddar cheese, the rate of softening of the PC samples was reduced, which can be attributed to an increase in the degree of emulsification as observed using CSLM. Multivariate data analysis correlated specific proteolytic events in the cheddar cheese with changes in textural and functional properties in the PC. This analysis clearly showed that the concentration of intact α_{s1}-CN in the cheddar cheese was strongly correlated with the decrease in hardness, fracturability, springiness, adhesiveness, and G' in the corresponding PC samples. Flowability increased in the PC samples and was correlated with the production of free amino acids in the cheddar cheese as well as development of pH 4.6 SN/TN. It was concluded from the multivariate data analysis that the degradation of α_{s1}-CN was the proteolytic event most strongly correlated with the softening seen in the PC samples during the first 28 d of ripening in the cheddar cheese base.

Acknowledgments

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