Monitoring the Chemical and Textural Changes During Ripening of Iranian White Cheese Made with Different Concentrations of Starter

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ABSTRACT

The effect of the concentration of starter inoculated to milk on the composition, free tyrosine-tryptophan content, microstructure, opacity, and fracture stress of Iranian White cheese (IWC) was studied during 50 d of ripening in brine. Three treatments of cheese were made using 1-fold (IWC1S), 2-fold (IWC2S), and 4-fold (IWC4S) concentrations of a direct-to-vat mesophilic mixed culture containing *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. *lactis* as starter. As ripening progressed, moisture and protein contents of the treatments continuously decreased, whereas their total ash, salt, and salt in moisture contents increased. Fat content and pH of cheeses remained stable during ripening. The pH of cheese milk at the time of renneting, which decreased by increasing the concentration of starter (6.57, 6.49, and 6.29 for IWC1S, IWC2S, and IWC4S, respectively), significantly affected most of the chemical characteristics and opacity of cheese. Lower pH values at renneting decreased moisture and ash contents, whereas cheese protein content increased. The concentration of free tyrosine-tryptophan in curd increased at first 29 d but decreased between d 29 and 49 of aging. The changes observed in cheese whiteness followed the changes in moisture content of the treatments. As the concentration of starter inoculated to milk increased, the value of fracture stress at a given ripening time significantly decreased, leading to a less resistant body against applied stress. A similar trend was also observed for fracture strain during cheese ripening. The micrographs taken by scanning electron microscopy provided a meaningful explanation for decrease in the value of fracture stress. As the cheese ripening progressed or the concentration of starter increased, the surface area occupied by the protein fraction in cheese microstructure decreased, leading the way to lower the force-bearing component in cheese texture.

Key words: Iranian White cheese, microstructure, rheology, starter culture

INTRODUCTION

The use of starter cultures containing lactic acid bacteria is an essential requirement in the manufacture of most cheeses (Cogan and Hill, 1993) including Iranian White cheese (IWC). Their major function is to produce lactic acid and, in some cases, flavor compounds (Fox et al., 2000). It is well known that reduction in milk pH due to acidification by starter cells or any other factor is accompanied by micellar demineralization (Banon and Hardy, 1992), which affects cheese curd strength (Cogan and Hill, 1993).

The basic composition and structure of cheeses are determined by the manufacturing operations like pH at renneting, but it is during ripening that the individual and unique characteristics of each cheese variety develop (Fox et al., 1993). Cheese proteolysis during ripening involves the concerted action of proteolytic enzymes. At the primary phase of ripening, enzymes such as residual chymosin and plasmin (an indigenous proteinase in milk) act on intact casein in the cheese curd. The further breakdown of casein, large peptides, and oligopeptides into small peptides and AA by the proteinases and peptidases of lactic acid bacteria forms the secondary phase of ripening (Mendia et al., 2000; Martínez-Cuesta et al., 2001; Attaie, 2005). These changes contribute in the development of flavor through release of free AA (O'Keeffe et al., 1976; Kondyli et al., 2003) and texture (Fox et al., 1993).

Iranian White cheese is a close-textured brined cheese, resembling Beyaz Peynir (Turkish White cheese) and Feta but differs from Feta in the way it is made. It is, for example, manufactured without dry salting of curd and slime formation on the curd surface before brining, which are essential for the development of the characteristic Feta flavor during ripening (Fox et al., 1993). At the industrial level, the ripening period is 40 to 90 d, but the cheeses made from raw milk in small rural production units may be ripened for 6 to 8 mo (Azarnia et al., 1997). Madadlou et al. (2005) studied the effect of fat content reduction and promoted proteolysis through increasing the concentration of rennet used during cheese manufacture on the textural properties of Iranian White cheese. As fat content in cheese decreased, storage

Received December 10, 2005.

Accepted March 29, 2006.

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modulus (G'), stress at fracture, Young's modulus of elasticity, and work performed up to fracture all increased. However, microstructure of cheese became compact, meltability decreased, and sensory properties of product became undesirable.

The objectives of the present paper were to 1) study the chemical and textural changes that occur in IWC during ripening, and 2) investigate the effect of the different concentrations of culture inoculated to cheese milk on these attributes.

MATERIALS AND METHODS

Treatments, Cultures, and Rennet

Three treatments of cheese were made as follows: cheese with 1-fold concentration of starter (**IWC1S**) made with 0.04 g of starter/kg of milk, cheese with 2-fold concentration of starter (**IWC2S**) made with 0.08 g of starter/kg of milk, and cheese with 4-fold concentration of starter (**IWC4S**) made with 0.16 g of starter/kg of milk. Cheeses were manufactured in triplicate, each replicate in 1 d using 7 kg of milk for each treatment. One lyophilized direct-to-vat mesophilic mixed culture (R-704, Chr. Hansen's Dairy Cultures, Hoersholm, Denmark) containing *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. *lactis* was used as starter. As coagulant, chymosin derived by fermentation of *Aspergillus niger* var. *awamori* [standard rennet, Chy-Max, Chr. Hansen Inc.; 183 International Milk Clotting Units (IMCU)/mL (International Dairy Federation, 1997)] was used at a concentration of 4.5 IMCU/kg of milk. Rennet was diluted 30-fold with cold water then added to each 7-kg batch of milk.

Cheese-Making Procedure

Fresh raw milk obtained from Animal Husbandry of Urmia University was batch-pasteurized at 65°C for 5 min (Hayaloglu et al., 2002) in a stainless steel container placed in a water bath, cooled to 35°C, and transported carefully to a cheese vat (model FT20-MkII, Armfield Ltd., Ringwood, Hampshire, UK). The milk was supplemented with 0.15 g of CaCl_2 /kg of milk and held at 35°C for approximately 60 min after inoculation of culture for starter maturation before the addition of rennet. The curd was cut crossways in cubes of 2 cm³ when firm (after approximately 55 min). After being cut, the curd was allowed to settle for 3 to 5 min and then gently agitated at a gradually increasing rate for 10 min to avoid fusion of freshly cut curd cubes and to facilitate whey expulsion. This was followed by whey draining and pressing the transferred curd into molds (14 × 13 × 25 cm) for 2.5 h (under the initial pressure of 0.3 kPa, which gradually increased up to approximately 2.9 kPa at the

first hour and held constant to the end of pressing) to complete draining. After pressing, the curd was cut in blocks (4 × 6 × 6 cm). The blocks were stored at 23 to 25°C for 19 to 20 h, placed in airtight plastic containers, and covered with 13% brine (brine was pasteurized beforehand at 80°C for 10 min, filtered through a clean cloth after rapid cooling, and pH adjusted to 4.65 by addition of 99% lactic acid). After sealing, the containers were stored first at 23 to 25°C for 24 h and then refrigerated at 5 to 6°C for the ripening period of 50 d.

Chemical Analysis

Titrateable acidity of milk was determined by the Dornic method, and its total solids were determined by drying 8 to 11 g of milk at 100°C for 5 h (Madadlou et al., 2006). The pH of milk and cheese samples was measured using a digital pH meter (microprocessor pH meter, model pH 537 WTW, Weilheim, Germany). Cheese was analyzed for moisture content by vacuum-oven (AOAC, 1997), salt content by Volhard (James, 1995), and ash content by dry ash method (AOAC, 1997). The fat content of milk and cheese samples was determined by the Gerber method (James, 1995), and their total protein contents were determined by measuring total nitrogen using the Kjeldahl method (AOAC, 1997) and converting it to protein content by multiplying by 6.38. All chemical measurements were done in triplicate or more. Cheese samples were chemically analyzed at d 2, 29, and 49 of ripening.

Apparent yield was calculated as the weight of cheese before brining (after 19 to 20 h storage at 23 to 25°C) divided by the weight of the milk used.

Quantification of Free Tyrosine-Tryptophan

Proteolysis rate in 2-, 29-, and 49-d-old IWC slurries was measured by determining tyrosine-tryptophan concentrations in TCA extracts following the method of Khosrowshahi (1988). Cheese slurries were prepared by mixing 2 parts of the grated cheese sample with 1 part of a sterile 5.2% solution of sodium chloride at 45°C. Duplicate 1.0-g samples of slurries were each dispersed in 5.4 mL of distilled water and placed in a 40°C water bath for 5 min. Ten milliliters of 12% (wt/vol) TCA solution was added to each suspension and allowed to stand for 10 min before being filtered through Whatman No. 2 filter paper. Five milliliters of each TCA extract was added to 10 mL of a solution containing 15% sodium carbonate and 2% sodium hexametaphosphate in Quickfit tubes kept in a 40°C water bath. This was followed by the addition of 3 mL of 3-time diluted Folin-phenol reagent to the tubes. Their contents were shaken thoroughly and were then held in a 40°C water bath for

5 min before measuring the absorbance at 650 nm by a UV-visible spectrophotometer (Ultrospec mode, LKB Biochrom, model 80-2092-26, Pharmacia, Cambridge, UK). To 6 mL of distilled water, 10 mL of 12% (wt/vol) TCA was added and kept at 40°C for 5 min, then filtered through Whatman No. 2 filter paper. The procedure was then followed by the addition of 5 mL of TCA filtrate and then 3 mL of Folin reagent to 10 mL of sodium hexametaphosphate solution. This solution was used as a blank. A tyrosine standard curve was prepared using concentrations of 0, 5, 10, up to 50 µg/mL in TCA filtrate.

Color Analysis

The color of 8-, 28-, and 50-d-old samples was quantitatively determined using a Hunter Lab system (model DP-9000, Hunter Associates Laboratory Inc., Reston, VA) in which the L value corresponds to whiteness. Color measurements performed in triplicate for each treatment at different sites.

Microstructure

Cheese samples were prepared for scanning electron microscopy at d 7, 29, and 49 of ripening following the method of Drake et al. (1996) with modifications. Cheese blocks were cut into approximately 5 to 6 mm³ cubes with a sharp razor and immersed in 2.5% glutaraldehyde fixative (Merck Science, Darmstadt, Germany) for 3 h. Cubes were then washed 6 times in distilled water (1 min each time), dehydrated in a graded (40, 55, 70, 85, 90, and 96%) series of ethanol for 30 min each, followed by defatting in 3 changes in chloroform (10 min each time). The defatted samples were kept refrigerated and covered with ethanol until they were freeze-fractured in liquid nitrogen (Sipahioglu et al., 1999) to approximately 1-mm pieces. These pieces were mounted on aluminum stubs by silver paint, dried to critical point, and coated with gold for 10 min in a sputter-coater (type SCD 005, Baltec Inc., Balzers, Switzerland). Samples were viewed in a scanning electron microscope (XL Series, model XL30, Philips, Eindhoven, the Netherlands) operated at 15.0 kV. Photomicrographs were recorded at 500× magnification and edited using ACD Photo Editor software, version 3.1 (Software Spectrum UK Ltd., High Wycombe, UK) for their exposure by maximizing gamma and contrast percentages.

Rheological Analysis

The simplest fundamental test, uniaxial compression (Tunick, 2000), was performed at d 7, 27, and 49 of ripening using a HTE Universal Testing Machine (S-Series Bench UTM model H5K-S, Hounsfield Test

Equipment Ltd., Redhill, UK) following the method of Tunick (2000) with modifications described by Madadlou et al. (2005) except that cheese blocks were cut into cylinders 24 mm in diameter and 16 mm high, and samples were compressed uniaxially with 62.5% deformation (10 mm) from the initial height of the sample in one bite. Calculated parameters were stress and Hencky strain at fracture (Madadlou et al., 2005). Each cheese was analyzed in triplicate.

Statistical Analysis

The experiment was replicated 3 times in a randomized complete block design, which incorporated the 3 treatments (IWC1S, IWC2S, and IWC4S). The ANOVA was carried out using the PROC GLM procedure of SAS statistical software package (Version 8.2, SAS Institute Inc., Cary, NC) to determine the effects of treatment of all variables. Duncan's multiple comparison test was used as a guide for pair comparisons of the treatment means. The level of significance was determined at $P < 0.05$.

RESULTS AND DISCUSSION

Chemical Composition

As expected, increasing the concentration of starter inoculated to milk significantly affected the pH value at renneting. The higher the initial starter concentration, the lower was the value of pH at renneting (6.57, 6.49, and 6.29 for IWC1S, IWC2S, and IWC4S, respectively). Table 1 shows the chemical characteristics of the milk used for cheese manufacture, treatment composition, yield of cheese making, and free tyrosine-tryptophan content as an index of proteolysis in curd. All of the measured attributes for cheese except fat content were altered by changing the starter concentration or by aging, or both, at 5°C. In agreement with the result obtained for fat content at the present study, Watkinson et al. (2001) reported no change for fat area distribution with pH and storage time for semihard model cheeses.

As ripening progressed, moisture and protein contents of the treatments continuously decreased, whereas their total ash, salt, and salt in moisture contents increased. When cheese is placed in brine, there is a net movement of NaCl molecules, as Na⁺ and Cl⁻, from the brine into the cheese because of the osmotic pressure difference between the cheese moisture and the brine. Consequently, the water in the cheese containing dissolved materials such as acids and minerals (calcium, magnesium, phosphorus, potassium, etc.) diffuses out through the cheese matrix with a flux approximately twice that of NaCl so as to restore osmotic pressure equilibrium (Guinee and Fox, 1993). The establishment of these dy-

Table 1. Mean ($\pm$ SD) of chemical composition, spectrophotometric absorbance, and cheese-making yield of cheeses made with different concentrations of starter¹

Item ²	Milk	IWC1S			IWC2S			IWC4S		
		7	28	48	7	28	48	7	28	48
Moisture, %	88.7 $\pm$ 0.29	54.82 ^a $\pm$ 0.21	52.93 ^b $\pm$ 0.41	51.90 ^c $\pm$ 0.28	53.31 ^b $\pm$ 0.23	51.53 ^c $\pm$ 0.29	50.63 ^d $\pm$ 0.44	51.64 ^c $\pm$ 0.23	50.69 ^d $\pm$ 0.36	50.01 ^e $\pm$ 0.31
Fat, %	3.19 $\pm$ 0.17	23 ^a $\pm$ 0.1	23 ^a $\pm$ 0.1	23 ^a $\pm$ 0.2	23 ^a $\pm$ 0.2	23 ^a $\pm$ 0.1	23 ^a $\pm$ 0.1	23 ^a $\pm$ 0.1	23 ^a $\pm$ 0.1	23 ^a $\pm$ 0.2
pH	6.64 $\pm$ 0.01	4.76 ^a $\pm$ 0.02	4.76 ^a $\pm$ 0.01	4.77 ^a $\pm$ 0.02	4.66 ^b $\pm$ 0.03	4.65 ^b $\pm$ 0.02	4.66 ^b $\pm$ 0.01	4.24 ^c $\pm$ 0.02	4.22 ^c $\pm$ 0.01	4.25 ^c $\pm$ 0.02
Protein, %	3.09 $\pm$ 0.01	17.96 ^d $\pm$ 0.18	16.62 ^f $\pm$ 0.11	16.14 ^g $\pm$ 0.17	18.52 ^c $\pm$ 0.19	17.41 ^e $\pm$ 0.21	16.58 ^f $\pm$ 0.19	20.06 ^a $\pm$ 0.12	19.12 ^b $\pm$ 0.26	17.41 ^e $\pm$ 0.18
M:P	—	3.05 ^c	3.18 ^b	3.21 ^a	2.87 ^e	2.95 ^d	3.05 ^c	2.57 ^f	2.65 ^f	2.87 ^e
Salt, %	—	3.97 ^c $\pm$ 0.02	4.16 ^b $\pm$ 0.02	4.29 ^a $\pm$ 0.01	4.00 ^c $\pm$ 0.02	4.15 ^b $\pm$ 0.03	4.29 ^a $\pm$ 0.2	3.98 ^c $\pm$ 0.02	4.14 ^b $\pm$ 0.01	4.30 ^a $\pm$ 0.02
Total ash, %	—	5.21 ^f $\pm$ 0.02	5.61 ^d $\pm$ 0.03	6.78 ^a $\pm$ 0.02	4.89 ^h $\pm$ 0.01	5.27 ^e $\pm$ 0.02	6.63 ^b $\pm$ 0.02	4.71 ⁱ $\pm$ 0.01	5.13 ^g $\pm$ 0.01	6.27 ^e $\pm$ 0.01
S/M, %	—	7.24 ⁱ	7.86 ^f	8.26 ^e	7.50 ^h	8.05 ^e	8.47 ^b	7.70 ^g	8.16 ^d	8.60 ^a
Tyrosine ³ (mg)	—	0.032 ^g $\pm$ 0.01	0.075 ^c $\pm$ 0.01	0.039 ^f $\pm$ 0.01	0.050 ^e $\pm$ 0.01	0.084 ^b $\pm$ 0.01	0.039 ^f $\pm$ 0.01	0.061 ^d $\pm$ 0.01	0.109 ^a $\pm$ 0.01	0.050 ^e $\pm$ 0.01

^{a-i}Means within the same row with different superscripts differ significantly ($P < 0.05$).¹IWC1S = Iranian White cheese with a 1-fold concentration of starter; IWC2S = Iranian White cheese with a 2-fold concentration of starter; and IWC4S = Iranian White cheese with a 4-fold concentration of starter.²M:P = Ratio of moisture to protein; S/M = salt to moisture ratio.³Tyrosine measured in a 5-mL aliquot.

namic phenomena decreased the moisture content of the treatments and increased their salt content as they ripened. Hayaloglu et al. (2002) related the decrease in protein content of Turkish White cheese (Beyaz peynir) during ripening to diffusion of some proteolysis products from the curd into the brine. In agreement with the findings in this study, Ehsani et al. (1999) reported that the amount of total N and NPN in the brine was increased during ripening of IWC.

Contrary to our results, Azarnia et al. (1997) reported a decrease in pH during ripening of IWC in brine (10% wt/vol, pH = 7.4), mainly because of completion of lactose fermentation and the liberation of amino and free fatty acids. The slow solubilization of colloidal calcium phosphate during ripening, which causes a slow increase in pH (Lucey et al., 2003), may have offset the tendency for a pH decrease in the present study, with the result that the cheese pH remained stable.

The value of pH at renneting significantly influenced the chemical characteristics of the treatments. As the acidification rate enhanced by increasing the concentration of inoculated starter to milk, the ash content of the treatments decreased, whereas their protein content increased. These trends were always the same at a given storage time. The decrease in the ash content was due to the solubilization of colloidal calcium phosphate, magnesium, and citrate ions (Le Graët and Gaucheron, 1999) at lower pH and a concomitant increase in the concentration of soluble calcium in the whey. The whey acts as a vehicle in which the soluble calcium is removed from the curd at whey drainage (Guinee et al., 2002).

Lower values of pH at renneting reduced the net charge on casein micelles and probably improved the activity of rennet (Guinee et al., 2002), leading to greater protein recovery in the curd. This, therefore, increased the protein content of cheese treatments. In agreement with the result obtained in the present study, Banks et al. (1987) reported that the amount of CP in cheese obtained from normally heat-treated milk (72°C for 16 s) acidified to pH 5.8 was higher than that of unacidified milk. As the pH at renneting decreased, the moisture content of the treatments decreased in the current study. Spangler et al. (1991) also reported that Gouda cheeses made from ultrafiltered milk with 3% starter had lower moisture than those with 1% starter. According to those researchers, the treatments with higher starter concentrations probably had increased whey syneresis and therefore lower moisture contents. This occurs because the rate of rearrangements of protein-protein bonds in the casein gels during their formation increases as pH drops (Watkinson et al., 2001).

Concentration of free tyrosine-tryptophan increased between d 2 and 29, whereas it decreased between d 29 and 49 of aging. Azarnia et al. (1997) reported the same

Figure 1. Whiteness changes of Iranian White cheese treatments during ripening. IWC1S, IWC2S, and IWC4S represent Iranian White cheeses made with 1-, 2-, and 4-fold concentrations of starter.

changes for concentration of free AA in IWC curd during the initial 50 d of aging. They hypothesized that the decrease could be due to decarboxylation, deamination, and transamination reactions. It is, however, our belief that the diffusion of free AA into the brine could be the main reason, as was explained by Carić (1993). As the concentration of starter inoculated to milk increased, the amount of free tyrosine-tryptophan in cheese increased at a given storage time. The strong probability of increased number of starter cells retained at the curd due to the increase in the initial number of cells used to inoculate the milk or decreased pH at wheying, or both, likely produced more proteinases and peptidases that were excreted into curds and caused more breakdown of casein molecules (Attaie et al., 2005) and larger peptides to smaller peptides and free AA (Dave et al., 2003). The amount of rennet retained in the curd was not measured in the current study; however, it could not take a part in evolution of differences between free tyrosine-tryptophan concentrations of the treatments at a given storage time. The portion of rennet lost in the whey during cheese making does not depend on pH at wheying in the case of microbial rennets (Holmes et al., 1977; Fox, 1989) such as chymosin derived by fermentation of *Aspergillus niger* var. *awamori*, which was used in the present study. The lower pH values of the treatments with higher starter may, however, somewhat improve the activity of retained rennet at the curd (Watkinson et al., 2001), leading to more hydrolysis of casein molecules.

Cheese Opacity

Figure 1 shows that the progressed ripening and increased concentration of starter significantly decreased

the whiteness of IWC. The scattering of light by any system is related to its heterogeneity (Pastorino et al., 2002). In a solid material such as cheese, light penetrates the superficial layers and is scattered by milk fat globules (Lemay et al., 1994) and whey pockets (Paulson et al., 1998). As ripening progressed, whey in serum pockets diffused from the cheese body out into the brine, as seen by moisture loss (Table 1). The surface area occupied by light-scattering centers was therefore decreased. Similarly to our results, Kaya (2002) reported that Gaziantep cheese samples ripened in the weakest brine had the highest moisture content and the highest L value compared with samples ripened in stronger brines. Color changes as a result of starter concentration were parallel to changes in the moisture content of the treatments at a given ripening time, which resulted in decreased light scattering, and hence, lower L values.

Microstructure and Rheological Analysis

The changes in microstructural features reflected the biochemical changes in IWC during ripening and also the effect of variations of starter concentration used in this study. The surface area occupied by the protein matrix (white areas in the scanning electron microscope micrographs) decreased visibly during ripening (as seen in panels from top to bottom in Figure 2). Similarly, as the concentration of starter increased, the surface area occupied by the protein matrix decreased at a given storage time (as seen in panels from left to right in Figure 2). The hydrolysis of cheese protein network and subsequent diffusion of small peptides and free AA to the surrounding brine may account for the microstructural changes that were observed during ripening and enhanced by increasing the concentration of starter. The protein matrix of the IWC4S treatment occupied the smallest surface area at the end of ripening (Figure 2).

Table 2 reports the values and trends of uniaxial compression parameters for IWC1S, IWC2S, and IWC4S during 49 d of ripening in brine at 5°C. The longer the ripening period, the lower were the values of stress and Hencky strain at fracture for a given treatment. Fat and moisture act as the filler in the casein matrix of cheese texture (Madadlou et al., 2005), giving it lubricity and softness. The casein matrix provides the elastic character to cheese texture. The decrease in the volume fraction of force-bearing component (protein) in cheese microstructure during ripening could account for most of the reduced firmness. It is noteworthy that diffusion of salt into cheese during aging changes the strength of the interactions between protein molecules by screening of charged groups. This reduces electrostatic repulsions between protein molecules but also lessens the number of plus-minus interactions between protein molecules

Figure 2. Scanning electron microscope micrographs of Iranian White cheeses (IWC); white areas indicate protein matrix: A) 7-d IWC with 1-fold concentration of starter (IWC1S); B) 27-d IWC1S; C) 49-d IWC1S; D) 7-d IWC with 2-fold concentration of starter (IWC2S); E) 27-d IWC2S; F) 49-d IWC2S; G) 7-d IWC with 4-fold concentration of starter (IWC4S); H) 27-d IWC4S; and I) 49-d IWC4S.

(Lucey et al., 2003). The domination of the latter effect likely weakened the structural bonds of the protein matrix of cheese, leading to less resistance against applied

stress. According to Kaya (2002), brines with less than 15% salt could cause weakening of cheese structure. In addition, the slow solubilization of colloidal calcium

Table 2. Mean ($\pm$ SD) of uniaxial compression parameters for Iranian White cheese with 1-, 2-, and 4-fold concentrations of starter (IWC1S, IWC2S, IWC4S, respectively)

Item	IWC1S			IWC2S			IWC4S		
	7	28	48	7	28	48	7	28	48
Fracture stress (kPa)	200.1 ^a $\pm$ 297.2	136.0 ^c $\pm$ 30.4	79.6 ^g $\pm$ 32.2	162.2 ^b $\pm$ 29.7	114.5 ^e $\pm$ 37.1	70.8 ^h $\pm$ 25.5	117.6 ^d $\pm$ 27.6	102.4 ^f $\pm$ 26.4	57.2 ⁱ $\pm$ 34.1
Fracture strain	0.52 ^a $\pm$ 0.03	0.40 ^d $\pm$ 0.01	0.31 ^f $\pm$ 0.01	0.47 ^b $\pm$ 0.02	0.33 ^e $\pm$ 0.02	0.30 ^g $\pm$ 0.01	0.44 ^c $\pm$ 0.02	0.31 ^f $\pm$ 0.02	0.30 ⁱ $\pm$ 0.03

^{a-i}Means within the same row with different superscripts differ significantly ($P < 0.05$).

phosphate undoubtedly had a part in softening the treatments during aging (Lucey et al., 2003).

Increasing the concentration of starter inoculated to milk also decreased cheese fracture stress and made the cheese body weaker. It also lowered the value of Hencky strain at fracture and shortened the cheese. The decrease in fracture stress clearly coincided with the decrease in surface area occupied by the protein fraction in cheese microstructure.

In the model of Horne (1998), micellar calcium phosphate is not just regarded as cross-links between casein nanoclusters (Tuinier and de Kruif, 2002), but also as a neutralizing agent, which, being positively charged, binds to negatively charged phosphoserine clusters to reduce the protein charge to the point at which the attractive interactions between hydrophobic regions of the casein can be allowed to dominate. The micelle is not dissociated at temperatures higher than 25°C (Banon and Hardy, 1992) by the dissolution out of colloidal calcium phosphate when the pH is decreased (Dalglish and Law, 1989; Lucey et al., 2003). It is due to the concurrent neutralization of the phosphoserine charge by the acid (Horne, 1998). The reduction in the amount of calcium associated with casein molecules would, however, increase electrostatic repulsion between caseins (Lucey et al., 2003) and cause a weakening of the structural bonds (Horne, 1998). This probably took a part in the decrease in value of stress at fracture as the pH at renneting decreased. Watkinson et al. (2001) similarly observed that fracture strain and stress of model cheeses increased as the pH increased.

CONCLUSIONS

The concentration of starter inoculated to milk caused variation in the value of pH at renneting, which in turn affected most of the chemical characteristics and opacity of IWC. The study indicated that texture (rheology and structure), chemical and biochemical composition, and opacity of IWC changed markedly during 50 d of aging in brine at 5°C. The changes occurred in microstructure of cheese because of casein hydrolysis, and subsequent diffusion of small peptides and free AA into the brine explained the decrease in cheese firmness due to ripening progress and increased concentration of starter inoculated to milk.

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