AN ABSTRACT OF THE THESIS OF

Sandra for the degree of Master of Science in Food Science and Technology presented on July 3, 2002.
Title: Sensory, Compositional and Texture Profile Analysis of High-Pressure Treated Fresh Renneted Cheese – Queso Fresco Style.

Abstract approved: ______________________
Lisbeth M. Goddik

A sensory method was developed to determine cheese texture by hand evaluation. Cheese sensory evaluation was conducted by panelists (n=8) on four commercial samples in duplicates. Standards, descriptors, methods of each attribute evaluation, sample size, and ballot were developed based on panelists' consensus. Fifteen total attributes, divided into five groups, were tested. Crumbliness was defined as the ease of the sample to break apart during manipulation using the thumb and two fingers for five times. Using Principal Component Analysis (PCA), four components were extracted with the first two explaining most of the variability (60.4%). PCA showed that moistness, crumbliness, color, cohesiveness, irregularity, and oiliness were the main attributes describing the samples. Irregularity and cohesiveness had 83.6% and -88.1% correlations with crumbliness, respectively. Panelists' performances were not significantly different ($p \leq 0.05$) and each subject used the method consistently for crumbliness. This method was then applied to evaluate and compare the sensory attributes of Queso Fresco.

Three types of Queso Fresco cheese were made: raw cheese (RC), High Hydrostatic Pressure (HHP) treated raw cheese (HP), and cheese made from HHP treated milk (HPM). Sensory attributes, compositions, microstructures and protein profile were compared. Sensory attributes were examined by ten trained panelists using hand evaluation method developed and instrumental methods (Texture

Profile Analysis (TPA) and 80% compression test). Protein, fat, and moisture contents were valuated by Micro Kjeldahl, Babcock, and Forced air draft oven respectively. Microstructure was examined by light microscopy using Acid Fuschin protein staining, while native and SDS PAGE were carried out to show the protein profile. One and eight days storage times were studied. HHP treatment of cheese or cheese milk (400 MPa, 20 min, ambient temperature) were shown to reduce microbial loads. HP and RC had similar microstructure, compositional (p-value$\leq$0.05), and sensory attributes, except color (p-value$\leq$0.05). HP and RC had distinct protein network, while HPM had a very diffuse network. HPM was different from both RC and HP. HPM was the least firm, least crumbly, most sticky and oily. HPM day one was firmer, less oily, less springy than day eight. HPM had higher moisture and yield, due to incorporation of denatured whey proteins, than RC and HP cheese.

The hand evaluation method developed was proven to be able to differentiate cheese textural attributes. Overall, HHP treatment of Queso Fresco produced cheese with similar characteristics as traditionally made Queso Fresco, while HHP treatment of cheese milk created cheese with weak texture characteristics. HHP treatment of cheese might be an alternative way to produce Queso Fresco with acceptable attributes and reduced microbial load.

Sensory, Compositional and Texture Profile Analysis of High-Pressure Treated Fresh Renneted Cheese – Queso Fresco Style

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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CONTRIBUTION OF AUTHORS

Dr. Marlene A. Stanford was involved with the data collection, data interpretation, and the writing of chapter 1 and 2.

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GENERAL INTRODUCTION

Cheese is considered as one way of preserving milk and increasing its economic and nutritional values. The United States production of cheese in 2000, excluding cottage cheese, was 8.25 billion pounds. This represented a 3.9% increase of production from 1999. Cheddar cheese was the most common product, accounting for 34.3% of total cheese production, while Hispanic style cheese constituted 1.2% of total cheese production (USDA Dairy Products 2000 Summary, April 2001).

Queso Fresco is fresh, soft, moist, crumbly, white cheese that originated from Latin American countries and is traditionally made from raw cow's milk (Clark et al., 2001). It has a mild, salty flavor, and is one of the favorite Hispanic-style cheeses with texture that softens but does not melt when heated. It can be used as a topping or filling in cooked dishes, commonly crumbled over salads, tacos, chili, burritos, and is good with fruits. Furthermore, it can be used as snacks with tortillas and cornbreads, or even as an ingredient in processed cheese (Torres, and Chandan, 1981).

Queso Fresco is traditionally made at home or at the farm, and has a shelf life under a week. Even though the basic ingredient is milk and direct acidification is commonly used, manufacturing processes are not standardized due to the geographic differences (Moore, Richter, and Dill, 1986). Depending on preference, some are pressed and some are not; acidification is achieved by the addition of fruit juice or vinegar, and rennet may be utilized. In addition, it can be made from whole milk, part skim, skim, cream or mixtures of them (Torres, and Chandan, 1981).

There is a substantial market for Queso Fresco and/or Hispanic style cheese in the United States due to the increasing ethnic diversity of the population. Other factors that might potentially increase the market of Hispanic style cheese, such as Queso Fresco, are the demands for new cheese flavors, new cheese varieties and products, the nutritional benefits due to its high mineral and protein contents, the economic advantages due to its relatively short or no ripening time, high yield and excellent functionality, such as not melting when heated (Torres and Chandan, 1981).

Due to FDA requirements commercially available Queso Fresco must be made from pasteurized milk. In 1985, 142 cases of *Listeria monocytogenes* infections were associated with contaminated Queso Fresco in Los Angeles County in California (Linnan et al., 1988). In 1997, home made raw milk Queso Fresco was implicated in the *Salmonella typhimurium* DT104 outbreak in Yakima County, Washington, which started the Abuela project (Bell, Hillers, and Thomas, 1999; and Villar et al., 1999).

There are commercially produced Queso Fresco, made from pasteurized milk available on the market. Even though it is a safe product, there are some concerns about the lack of acceptable flavor and texture, particularly crumbliness. It is perceived that sensory characteristics, especially texture, of pasteurized Queso Fresco are inferior to those of raw milk Queso Fresco.

Pasteurization is widely used to reduce the microbial load of raw milk, thus, providing safer cheese. However, pasteurization may damage milk's cheese making properties. Pasteurization may negatively affect cheese flavor and flavor development as compared with raw cheese due to inactivation of enzymes needed for flavor development (Fox et al., 2000a).

Corredig and Dalgleish (1996) found that heat treatment of milk caused interaction between denatured whey proteins, especially α-lactalbumin (α-La) and β-lactoglubulin (β-Lg), with casein micelles. Hence, cheese milk pasteurization might cause a change in the cheese making properties of the milk (such as, increased yield and moisture retention). In their study, the interaction rate was affected by the time and temperature, the heat transfer of the heating medium to the milk and also the type of heating method employed. In HTST (High Temperature Short Time) pasteurization, there were some α-lactalbumin/k-casein complexes found and a higher amount of β-lactoglubulin/k-casein complexes were formed than in raw milk, which may cause a defect in cheese texture. Birkkjaer et al. (1961) and Lau et al. (1990) also found that pasteurization of cheese milk has been shown to make cheese with lower firmness due to higher moisture compared with raw milk cheese.

High hydrostatic pressure processing (HHP) is an alternative method to heat treatment that is gaining popularity. HHP does not use heat; instead, it uses a liquid medium to transfer pressure equally from all directions to the food. Numerous projects have shown that HHP reduces microbial load while causing no or only minor changes in nutritional properties and flavor compounds. HHP inactivates bacteria by changing the properties of the cell membrane. Generally, yeasts and molds are most sensitive to the effect of HHP, gram positive bacteria are more resistant to HHP than gram negative types, and bacterial spores are the most resistant (O'Reilley et al., 2000).

Numerous studies have been done to investigate the effect of HHP on microbial load either in foods, buffers or growth medium (Timson, and Short, 1965; Sale, Gould, and Hamilton, 1970; Metrick, Hoover and Farkas, 1989; Hoover et al., 1989; Knorr et al., 1992; Cheftel, 1995; Earnshaw, 1995; Heinz and Knorr, 1996; Patterson, and Kilpatrick, 1998; Kalchayanand et al., 1998; Raso et al., 1998; Gervilla et al., 1999; Alpas et al., 1999; Benito et al., 1999; Reddy et al., 1999; Ellenberg, and Hoover, 1999;Gervilla, Ferragut, and Guamis, 1999; Garcia-Graells, Valckx, and Michiels, 2000; O'Reilly et al., 2000; and Linton, McClements, and Patterson, 2001).

HHP can cause the dissociation of acid groups of amino acid side chains; thus, in the presence of oxygen, free –SH groups may be oxidized to disulfide bonds. It appears that high molecular weight food components, with higher structures that are important for functionality and structure determination, are sensitive to pressure. By contrast, low molecular weight components, which are responsible for nutritional and flavor characteristics, are less affected by pressure (Cheftel, 1992, Tewari et al., 1999).

Drake et al. (1997) demonstrated that Cheddar cheese made from high pressure treated milk (345 MPa or 586 MPa for 15 min) tended to produce cheese with higher moisture and wet weight yield than using either pasteurized or raw milk. The higher moisture content of pressurized milk Cheddar cheese causes a defect in texture, which might be beneficial for low fat cheese. The microbial load reductions were shown to be similar between pressurized and pasteurized cheese milk. There were no detrimental effects by high pressure treatment of cheese milk observed on the cheese flavor. The overall structure of cheese made from pressurized milk was similar to that of raw cheese, while pasteurized cheese had smaller fat globules. HHP treatment of cheese milk at 300-400 MPa has been found to cause an increase in cheese yield in conjunction with additional beta lactoglobulin and moisture retention, as well as an increase in milk coagulation properties (Lopez-Fandino, Carrascosa, and Olano; 1996). Whey proteins, especially beta lactoglobulins, are progressively denatured as pressure is increased. The denatured whey protein is incorporated into the curd (rennet gels), which may contribute to the increase in water binding properties (Needs et al., 2000).

Molina et al. (2000) have demonstrated that batch pasteurization of milk induced only slight β-lactoglobulin denaturation, while HHP caused extensive denaturation. Low fat cheese made from pasteurized milk had more β-lactoglobulin in the whey then cheese made from pressurized milk, indicating that more whey proteins were retained in the pressurized milk cheese. Texture Profile Analysis (TPA) showed that cheese made from pressurized milk had lower degrees of firmness, gumminess and chewiness than cheese made from pasteurized milk. There were no significant differences observed in cohesiveness and springiness.

Cheese made from pressurized milk had higher taste, texture, and overall acceptability score than cheese made from pasteurized milk, as determined by sensory analysis.

Pressurized milk Cheddar cheese was reported to have slightly less fat content, higher moisture, higher protein content, higher fat to dry matter ratio and slightly higher yield compared with pasteurized milk cheese (Kheadr et al., 2002). Pressurization of milk increased the cheese moisture content significantly due to changes induced by HHP on the milk proteins, which increases the water binding capacity and also possible syneresis reduction. Pasteurization was shown to be more effective in reducing total bacteria and *Listeria* viable counts in milk than HHP, however the overall microbiological quality was not significantly different between the fresh and 3 months old pasteurized and pressurized milk cheese. Pressurized milk cheese exhibited higher TPA values for firmness, cohesiveness, and fracturability compared with pasteurized milk cheese. Transmission electron microscopy (TEM) demonstrated that HHP treatment on milk negatively affects casein and fat structures. HHP caused reduction in casein micelles and fat globules sizes, and also disintegrated casein particles, which might induce casein-fat and/or casein-casein interaction. A very compact matrix constructed of mainly small casein micelles with evenly distributed, uniform, and very small fat globules was obtained in pressurized milk cheese compared with pasteurized milk cheese, in which matrix contained large casein micelles with individual, irregular, and unevenly distributed fat globules.

Messens et al. (2000) studied the effect of HHP treatment (50-400 MPa, 1 hour) on Gouda cheese. There were no moisture and proteolysis rate changes observed. These investigators found that the hydrophobic interactions were weakened by the HHP treatment, which lead to paracasein network structural changes that caused HHP treated cheese to have less rigid, less solid-like and more viscoelastic properties than untreated cheese. The fat globules size were unaffected by the HHP treatment. During ripening, the treated cheese began to resemble untreated cheese, suggesting reversible protein denaturation. Dissolution experiments designed to show the chemical bonds that stabilize the cheese networks and subsequently broken by HHP treatment were carried out. The results suggested that intermolecular disulfide bonds made no contribution to cheese network stabilization, possibly because the major caseins contain neither cysteine nor cystine, thus lacking the ability to form disulfide bonds inter- or intramolecularly. Hydrophobic interactions were found to be important in stabilizing the cheese protein network in untreated cheese. However, these kind of interactions were decreased when cheese was treated at a pressure below 400 MPa and were absent at 400 MPa. In both treated and untreated cheeses, electrostatic and perhaps hydrogen bonds were found to be essential in stabilizing the protein network.

High pressure treatment of cheese was done primarily to obtain faster ripening (O'Reilley, 2001). There is limited research on high pressure treatment of cheese that focuses on cheese texture.

Texture is one of the more critical factors in determining consumers' acceptability of a given cheese type. Texture can be evaluated by sensory analysis and/or instrumental techniques. One of the most commonly used sensory analyses is Quantitative Descriptive Analysis (QDA) that consists of three main steps, namely: vocabulary development, intensity measurement, and data analysis. Usually, 6-12 people are screened and trained before the conduct of sensory evaluation to familiarize panelists with the terms and the scale used. The terms to be evaluated, the references, the intensity of the reference, and the sequence on evaluating each attribute are decided by the panelists' consensus. QDA can be deployed to describe the product sensory characteristics ranging from initial visual assessment to aftertaste, or to simply focus on a narrow range of attributes (Lawless, and Heyman, 1999).

A widely used instrumental test for texture measurement is Texture Profile Analysis (TPA), which attempts to imitate the action of human jaw during mastication. The instrument compresses the sample twice in a reciprocating motion with a pause time between compressions, then measures the required force during the compression-decompression cycles, and plots the data as a force-time graph. The resultant graph represents an integral presentation of the textural characteristics of the sample (Friedman et al., 1963).

TPA measured eight textural parameters, namely: firmness (the force peak at the first compression cycle), fracturability (the force at first significant break in the curve on the first bite), cohesiveness (the ratio of the positive force areas under the first and second compressions), adhesiveness (the negative force area for the first bite), springiness (the distance that the sample recovered during the interval time between the first and second bite), gumminess (the product of hardness, and cohesiveness), chewiness (the product of hardness, cohesiveness, and springiness), and resilience (the ratio of the positive force areas (A1/A2) under the first compression, where A1 is the area from the first point when the probe touches the sample to the first probe reversal point, and A2 is the area from the first probe reversal to the point where the force returns to zero) (Bourne, 1976).

Crumbliness is a desired textural attribute of Queso Fresco (Hwang and Gunasekaran, 2001). Consumers usually crumble these cheeses prior to use and evaluate crumbliness during breaking. Thus a method that would measure crumbliness by hand would seem to be more appropriate than an oral approach as commonly done in research. Few methods are available for hand evaluation descriptive sensory analysis, and they are usually only suitable for specific cheese types.

Hwang and Gunasekaran (2001) have proposed an objective method on measuring the level of crumbliness in Queso Fresco type cheeses using Texture Profile Analysis (TPA), uniaxial compression, followed by particle size analysis of the compressed cheese, and shear fracture tests. They conducted descriptive sensory profiling and rated five attributes: moistness, firmness, crumbliness, particle size, and particle size uniformity. Sensory crumbliness was best correlated with the number of particles obtained from the particle size analysis of the compressed cheese. Drake et al (1999) has shown that by mouth and by hand terms are highly correlated, and both methods of evaluation can be used to describe and differentiate cheese texture.

The objectives of this study were to propose a method of non-oral evaluation descriptive panel to measure cheese texture, with emphasis on crumbliness and to investigate whether HHP treated raw cheese or cheese made from HHP treated milk provide cheese with textural characteristics similar to raw milk fresh Queso Fresco cheese.

CHAPTER 1

METHOD DEVELOPMENT OF MEASURING CHEESE CRUMBLINESS USING HAND EVALUATION: PRELIMINARY STUDY

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ABSTRACT

Cheese sensory evaluation was conducted by panelists (n=8) on four samples in duplicates. Standards, descriptors, methods of each attribute evaluation, sample size, and ballot were developed based on panelists' consensus. Fifteen total attributes, divided into five groups, were tested. Crumbliness was defined as the ease of the sample to break apart during manipulation using the thumb and two fingers for five times. Using Principal Component Analysis (PCA), four components were extracted with the first two explaining most of the variability (60.4%). PCA showed that moistness, crumbliness, color, cohesiveness, irregularity, and oiliness were the main attributes describing the samples. Irregularity and cohesiveness had 83.6% and -88.1% correlations with crumbliness, respectively. Panelists' performances were not significantly different ($p \leq 0.05$) and each subject used the method consistently for crumbliness.
Keywords: cheese, crumbliness, descriptive, hand evaluation, and texture

INTRODUCTION

Texture is an important attribute determining quality and consumers' acceptance of cheese. Texture is defined as "the attribute of a substance resulting from a combination of physical properties, that may include size, shape, number, nature and conformation of constituent structural elements, and perceived by the senses of touch (kinesthesia and mouthfeel), sight and hearing" by the British Standards Institution (Brennan, J.G., 1988). Based on this definition, texture is a sensory characteristic that can be evaluated by the sense of touch, sight and hearing.

Lee and colleagues (1978) have determined important texture attributes of cheese by gathering data from questionnaires and oral sensory evaluation on six types of cheese, while also relating those attributes with compression tests using an Instron Universal Testing Machine. From the questionnaires, firmness, softness, creaminess, chewiness, smoothness, mixing properties, elasticity, and melting were attributes liked in cheese; while crumbliness, graininess, plastic, mushy, gooey, runny, and waxy were disliked.

Crumbliness is a desired textural attribute of some types of cheese, such as Feta and Queso Fresco. Consumers usually crumble these cheeses prior to use and evaluate crumbliness during the breaking process. Thus a method to measure crumbliness by hand is more appropriate than orally as typically done in research. Few methods are available for hand evaluation descriptive sensory analysis and they are usually only suitable for specific cheese types.

Hwang and Gunasekaran (2001) have proposed an objective method for measuring crumbliness of Queso Fresco type cheeses using Texture Profile Analysis (TPA), uniaxial compression, followed by particle size analysis of the compressed cheese, and shear fracture tests. They conducted a descriptive sensory profiling and rated five attributes: moistness, firmness, crumbliness, particle size, and particle size uniformity. Sensory crumbliness was best correlated with the number of particles obtained from the particle size analysis of the compressed cheese. Drake et al (1999) has shown that mouth and hand terms were highly correlated, and both methods of evaluation can be used to describe and differentiate cheese texture.

The American Dairy Science Association, long ago developed a scorecard to grade cheese quality that includes cheese flavor, color, body and texture. Depending on the cheese type, different attributes are evaluated. For Cheddar cheese, nine attributes are graded in the body and texture part of the scorecard, namely: corky, crumbly, curdy, gassy, mealy, open, pasty, short, and weak, which are evaluated by sight and touch. Non-oral evaluation of cheese texture can avoid taste and olfactory fatigue. The scorecard is intended for grading and/or contest purposes and score (from 0 to 5, where 5 = no criticism) is given based on defects observed. It is not anchored to any standards, and the scorecard is only available for certain cheese types, such as Cheddar (Bodyfelt, F.W., et al, 1988). Even though it evaluates texture attributes by sight and touch, it is not a descriptive analysis. By descriptive analysis, more information is obtained, while also allowing for more statistical analysis.

The objective of this study was to propose a non-oral evaluation descriptive panel method to measure cheese texture, with emphasis on crumbliness.

MATERIALS AND METHODS

Cheeses

Commercial cheeses for standards and samples were purchased at local grocery stores (Corvallis, OR). Cheeses chosen for the standards were Lorraine Swiss, Emmentaler Swiss, Big Cut Parmesan, Extra Sharp Cheddar, Mild Cheddar, Low Moisture Part Skim Mozzarella, Whole Milk Mozzarella, American, Velveeta, Cream Cheese, and Feta. Cheeses used for testing were Feta, two brands of Queso Fresco and Monterey Jack. The Feta cube for the standard was wetted by either 5 drops of the whey from within the package or by water.

Cheeses were stored at refrigeration temperature (4-5°C) until use. The cheeses were cut into 1.5x1.5x1.5 cm^3 cubes using cheese cutter and prepared on the same day as training and testing. Standard cheeses were served on plates wrapped with plastic saran wrap, while the samples were served in individual plastic cups with lids coded by 3-digit random numbers. Samples and standards were taken out from the refrigerator one hour before presentation to equilibrate to room temperature. Each of the four samples was presented in duplicate. The order of sample presentation was randomized.

Sensory evaluation

Panelists were selected based on interest and familiarity with cheese. A total of ten subjects participated in the method development, but only eight of them were available on the testing day. Six-one hour training sessions were conducted. A 16-point intensity scale was used, where 0 = none and 15 = very extremely present. The first three sessions were used to describe and determine attributes, sample size, standards, procedure of evaluating each attribute, and the ballots until reaching consensus. Three cube sizes of cheese were introduced to the subjects: 1.5x1.5x1.5 cm^3, 1.8x1.8x1.8 cm^3, and 2.0x2.0x2.0 cm^3, and the smallest cube size was chosen. Panelists were exposed to different cheeses and asked to tell and describe the attributes pertinent to the cheeses before, during, and after breaking/crumbling, to choose references from the cheeses and assign intensity anchors for each attribute.

The terms, anchors and cheese standards chosen are shown on table 1. A total of fifteen attributes were obtained and categorized into five groups: appearance before breaking, texture before breaking, texture during breaking, residuals left after breaking, and appearance after breaking.

Standards chosen for the "appearance after breaking," shown on figure 1, are as follows: B=1.5x1.5x1.5 cm^3 cube of Feta chopped for 3 seconds (average particle diameter size was about 6.6 mm); C= 1.5x1.5x1.5 cm^3 cube of Parmesan grinded for 10 seconds (average particle diameter size was about 5 mm); D= 1.5x1.5x1.5 cm^3 cube of Parmesan grinded for 20 seconds (average particle diameter size was about 1.5 mm); G= 2.0x2.0x2.0 cm^3 cube of mild Cheddar crumbled by hand five times (average particle diameter size was about 17 mm); H= 1.5x1.5x1.5 cm^3 cube of Cream cheese crumbled by hand five times (average particle diameter size was about 13 mm). Average diameter was an average of five curds after manipulation, except for cream cheese (average of all (3) particles). Blender used was Osterizer 10 cycle blend pulsematic. The rest of the sessions were used to familiarize the subjects with the attributes, standards, evaluation, and the ballot used to minimize variability among subjects.

Panelists evaluated eight samples non-orally in individual booths under normal light conditions on testing day. Panelists were provided with plates and moist napkins to cleanse their hands after each sample and before the first sample.

Samples were presented at room temperature. Each panelist was also provided with the cheese standards, a ballot, intensity anchors, and pictures of the "appearance after breaking" standards in original size.

Statistical analysis

Analysis of variance (ANOVA) was applied to the attributes tested individually with significance level at p-value$\leq$0.05 to evaluate panelists' performance and if any of the samples were different. Principal Component Analysis (PCA) was employed to see which attributes were important in describing and differentiating the samples. A correlation matrix was conducted to determine if any of the attributes correlated. Sample comparison was determined by Tukey-HSD to include all possible sample combinations. All statistical analysis was performed with SPSS statistical package (SPSS version 10.1, SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSIONS

PCA (Table 2) extracted four components (explained 77.8% variance) with the first two components explaining most of the variability (60.4%). Component one consisted of color, moistness, oiliness, irregularity, crumbliness, cohesiveness, wet residual, oil residual, piece size and the number of piece after breaking. Component two consisted of springiness, stickiness, sticky residual, oil residual, and piece uniformity after breaking. These two components covered almost all of the attributes tested, except firmness, which is in component 3 (Table 2). PCA showed that the attributes are effective in describing the samples, especially moistness, crumbliness, color, cohesiveness, irregularity, and oiliness. Figure 2 showed the plot of attributes on components 1 and 2 for the cheese samples tested. It showed that the designated attributes could differentiate the cheese tested. Monterey Jack was separated from the other three samples. Queso Fresco and Feta could be differentiated by attributes in component 2. Even the two

Table 1.1 Attributes, Definitions and Anchors Determined and Used by Panelists For Cheese Texture.

GROUP Procedure	ATTRIBUTE	DEFINITION	ANCHOR (0=none, 3=slight, 7=moderate, 12=extreme)
Appearance Before Breaking Please take a look at the sample and rate	Color	From white to yellow	1=Cream cheese 2=Feta 7=Low moisture, part skim Mozzarella 14=Emmentaler Swiss
	Moistness	From dry to moist	2=Extra sharp cheddar 4=Low moisture, part skim Mozzarella 14=Feta
	Surface oil (Oiliness)	From dry to oily based on the presence of oil droplets on the surface	2=Mild Cheddar 7=Low moisture, part skim Mozzarella 14=Emmentaler Swiss left out at room temp for one day
	Surface irregularity (irregularity)	From regular/smooth to irregular/bumpy, including cracks, curds, and holes on the surface	2=Low moisture, part skim Mozzarella 12=Feta 14=Lorraine Swiss
Texture Before Breaking Please lift the sample from plate and press the sample gently	Firmness	From soft to firm and is defined as the force required for compressing the cheese with the fingers	3=Cream cheese 7=Whole milk Mozzarella 10=Emmentaler Swiss 15=Parmesan

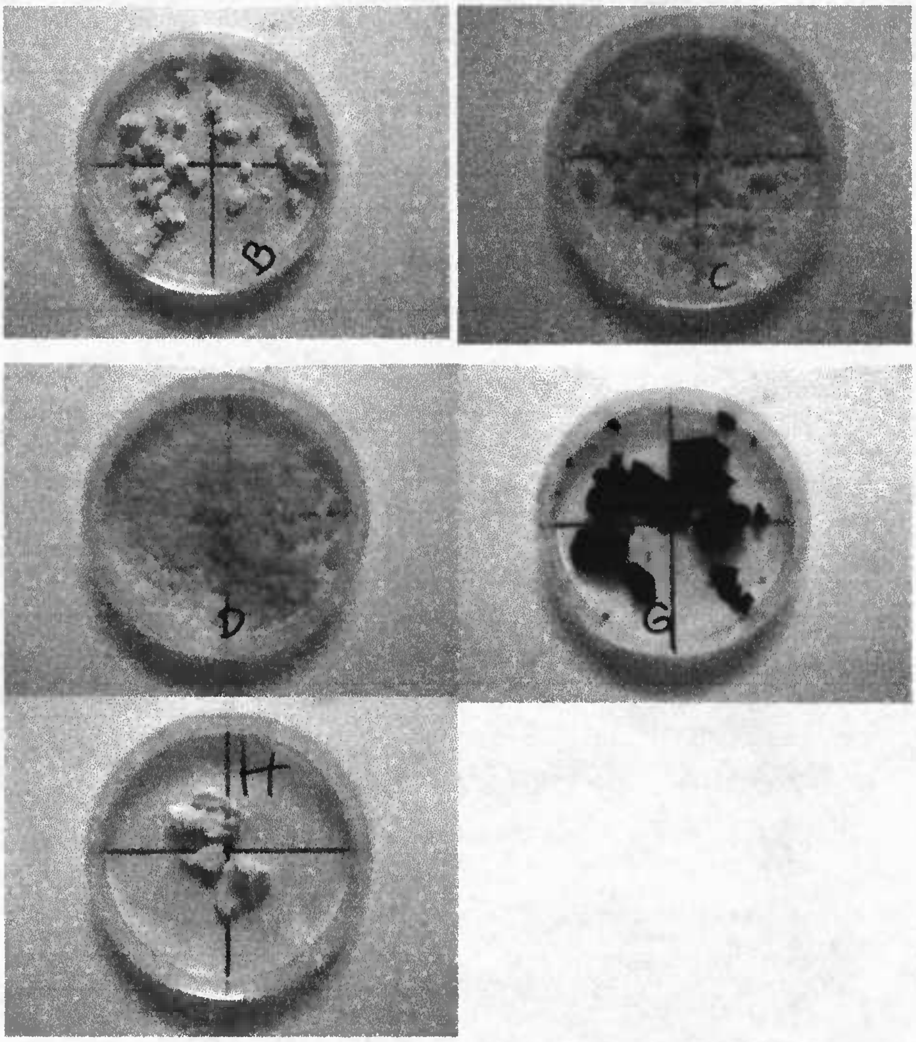
Table 1.1 (Continued)

for 1-2 seconds without breaking it and rate	Springiness	From not springy to springy and is defined as the rate of the cheese springs back to original shape/height after compressed	0=Cream cheese 7=Whole milk mozzarella 10=Emmentaler Swiss
Texture during breaking Please manipulate the sample using thumb and two fingers five times and rate	Crumbliness	From not crumbly to crumbly and is defined as the ease of the sample to break apart and crumble during manipulation	0=Cream cheese 5=Extra sharp Cheddar 6=Emmentaler Swiss 12=Feta
	Stickiness	From not sticky to sticky and is defined as how the sample sticks to the fingers during manipulation	3=Emmentaler Swiss 9=Feta 11=Extra sharp Cheddar 14=Cream cheese
	Cohesiveness	From not cohesive to cohesive and is defined as how well the sample holds together during manipulation	3=Feta 5=Emmentaler Swiss 9=Whole milk Mozzarella 14=Velveeta
Residuals Left After Breaking Please feel any residual (left over) tactile feeling on the fingers and rate	Oiliness/greasiness residual	From not oily to oily and is defined as how much residual oil/grease/fat film is left on the fingers after manipulation	3=Whole milk Mozzarella 11=Extra sharp Cheddar 13=Cream cheese

Table 1.1 (Continued)

	Sticky residual	From no curd/cheese sticks to the hand to a lot stick on the hand and is defined as how much curd or cheese particle left on the fingers after manipulation	4=Extra sharp Cheddar 9=Feta 14=Cream cheese
	Wet residual	From dry to wet and is defined as how much moisture or water felt on the fingers after manipulation	2=Extra sharp Cheddar 5=Whole milk Mozzarella 12=Feta
Appearance after breaking Please look at the sample after manipulation and rate	Particle size	From very small to large and is defined as the average particle size observed after the sample is manipulated	2=D 7=B 13=G
	Number of pieces	From very few to a lot and is defined as how many particles observed on the plate after sample manipulation	2=H 5=B 12=C 14=D
	Particle size uniformity	From not uniform to uniform and is defined as how uniform the particle size after sample manipulation	4=C 8=G 13=B

Figure 1.1 Standards used for "appearance after breaking." Attributes included are: particle size, number of pieces, and particle size uniformity.



brands of Queso Fresco could be separated by component 2 attributes. From figure 2, Queso Fresco B seemed to have higher springiness than Queso Fresco A, which was supported by the Tukey-HSD analysis on springiness with a 95% confidence level.

Feta cheese was described as more sticky, with higher sticky and oil residual, and had more uniform piece size after manipulation than the two brands of Queso Fresco. Both Queso Fresco were springier than feta. Feta and the two Queso Fresco brands had similar firmness, irregularity, moistness, crumbliness and wet residual (Figure 2).

Monterey Jack was more cohesive, with more yellow color, more oil residue and bigger piece size after manipulation than the other three cheese samples (Figure 2). Figure 2 indicated that the replications are close, which meant that each panelist evaluated the samples consistently.

Figure 2 showed the correlation among the descriptors. In component 1: moistness, irregularity, wet residual, crumbliness and number of pieces were positively correlated with each other. All of those attributes were negatively correlated with color, oiliness, cohesiveness, oily residue and piece size. In component 2: stickiness, sticky residue, oil residue and uniformity were positively correlated with each other while all were negatively correlated with springiness. Table 3 supported the results and displayed the actual correlation coefficient value.

Crumbliness is a complex texture attribute and its evaluation might be correlated with other attributes. The results indicated that crumbliness had 87.7% and 83.6% positive correlation with moistness (appearance) and irregularity (appearance), respectively. Crumbliness was also negatively correlated with cohesiveness (-88.1%).

Figure 3 showed that the panelists had the same trend in evaluating the samples for crumbliness, which indicated that the panelists could use the standards and attributes to differentiate the samples. Each panelist was consistent

Table 1.2 Attributes Tested and Component Associated as Extracted by Principal Component Analysis (PCA).

ATTRIBUTE	COMPONENT 1	COMPONENT 2	COMPONENT 3	COMPONENT 4
Color	-0.93	0.10	-4.93E-2	0.17
Moistness	0.94	7.24E-2	-4.21E-2	-0.13
Oiliness	-0.80	4.8E-2	9.30E-2	0.26
Irregularity	0.87	0.34	-0.13	1.28E-2
Firmness	0.34	3.77E-2	0.55	0.66
Springiness	-0.24	-0.50	0.48	2.60E-3
Crumbliness	0.93	2.93E-2	-6.97E-3	7.71E-2
Stickiness	-1.92E-3	0.77	0.49	-0.16
Cohesiveness	-0.92	1.79E-2	3.70E-2	-0.13
Sticky residual	0.19	0.66	0.46	-0.20
Wet residual	0.65	0.22	-0.44	-4.92E-2
Oil residual	-0.54	0.50	4.16E-2	-0.18
Piece size	-0.76	0.32	-0.16	0.17
Number of piece	0.78	-0.22	0.36	0.15
Uniformity	9.17E-3	0.49	-0.43	0.57
Variance Explained	46.4%	14.0%	10.3%	7.0%

Figure 1.2 PCA plot of first two Principal Component (PC) scores anchored with descriptors. The circles show samples that are significantly different (p-value ≤ 0.05 by Tukey-HSD) on PC 1 or PC 2 scores.

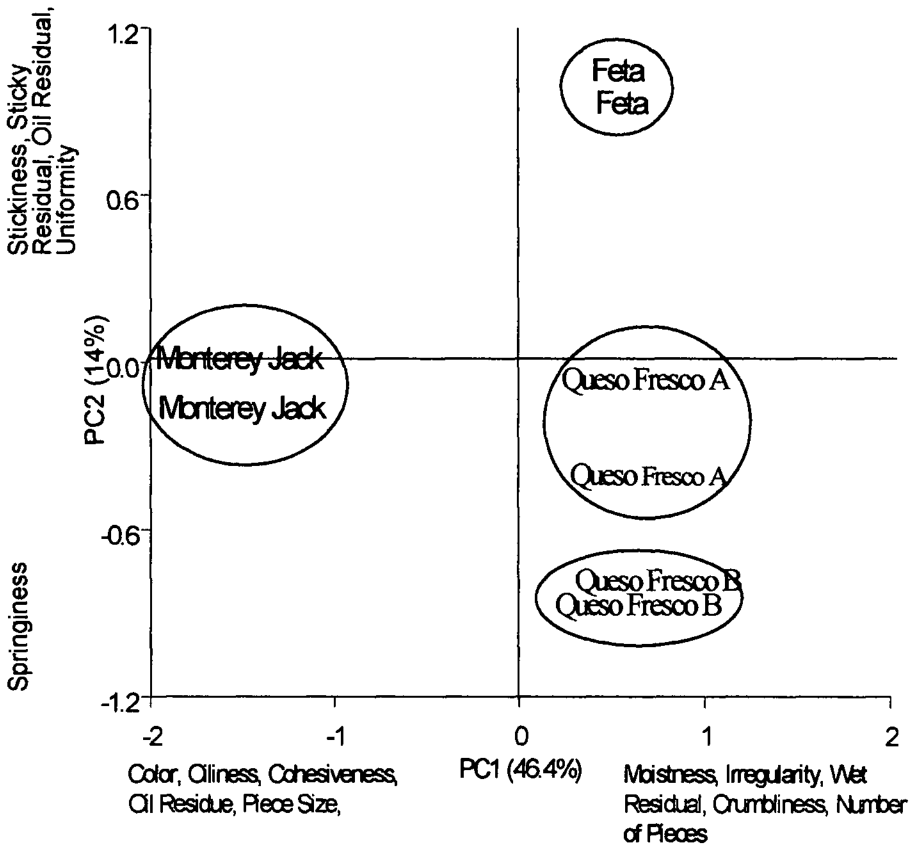
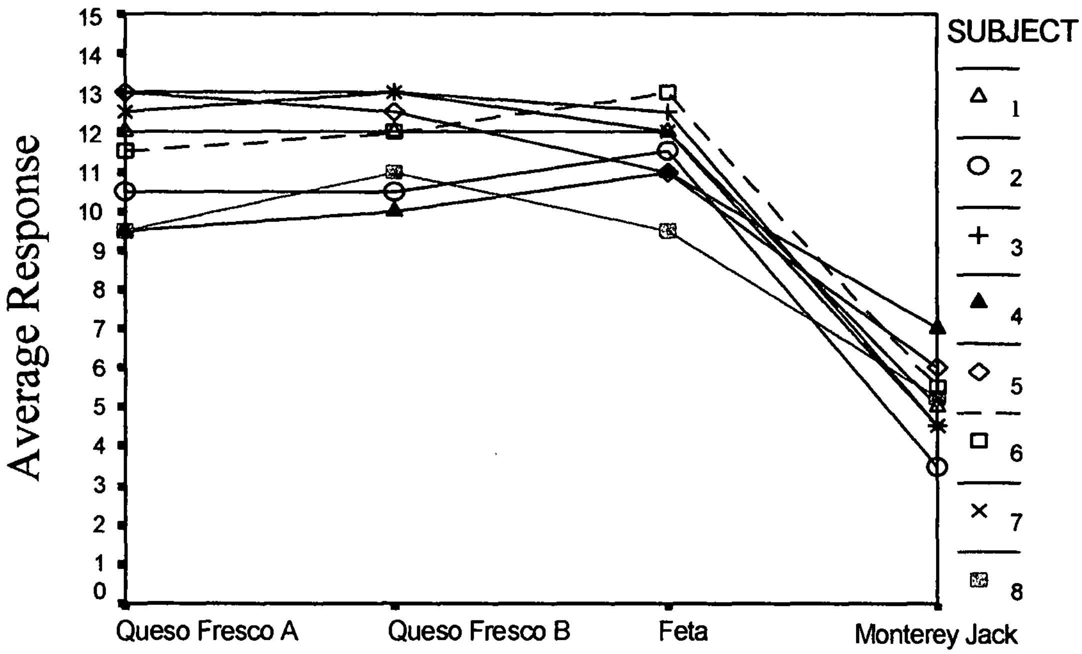


Table 1.3 Correlation Coefficients of All Attributes.

	1[a]	2[a]	3[a]	4[a]	5[a]	6[a]	7[a]	8[a]	9[a]	10[a]	11[a]	12[a]	13[a]	14[a]	15[a]
1[a]	1.00	-0.85	0.79	-0.77	-0.26	0.16	-0.84	0.04	0.80	-0.14	-0.56	0.47	0.82	-0.71	0.07
2[a]		1.00	-0.81	0.86	0.18	-0.24	0.88	0.09	-0.85	0.20	0.58	-0.48	-0.66	0.67	0.09
3[a]			1.00	-0.65	-0.08	0.13	-0.65	0.01	0.68	-0.17	-0.50	0.55	0.56	-0.45	-0.02
4[a]				1.00	0.27	-0.45	0.84	0.19	-0.79	0.24	0.62	-0.22	-0.53	0.53	0.26
5[a]					1.00	0.07	0.35	0.17	-0.35	0.15	-0.01	-0.20	-0.21	0.46	0.06
6[a]						1.00	-0.19	-0.15	0.20	-0.07	-0.31	-0.06	0.06	0.03	-0.29
7[a]							1.00	0.01	-0.88	0.14	0.65	-0.44	-0.65	0.72	0.10
8[a]								1.00	0.07	0.64	-0.07	0.37	0.18	0.01	0.08
9[a]									1.00	-0.11	-0.60	0.45	0.62	-0.75	-0.13
10[a]										1.00	0.13	0.11	-0.06	0.10	0.13
11[a]											1.00	-0.19	-0.31	0.30	0.27
12[a]												1.00	0.45	-0.48	0.00
13[a]													1.00	-0.74	0.19
14[a]														1.00	-0.10
15[a]															1.00

[a] 1=color, 2=moistness, 3=oiliness, 4=irregularity, 5=firmness, 6=springiness, 7=crumbliness, 8=stickiness, 9=cohesiveness, 10=sticky residue, 11=wet residue, 12=oil residue, 13=piece size, 14=number of piece, 15=uniformity.

Figure 1.3 Subjects' average responses for crumbliness for all samples. Subjects used 16-point intensity scale (0 = none, 3 = slight, 7 = moderate, 11 = high, 12 = extreme).



in using the anchors and standards. The panelists seemed to use a different part of the scale (the range is about 4 points from the highest to lowest mean score) due to inherent differences in the subjects. This variability would be expected to diminish with additional training. From the ANOVA results for crumbliness, it was evident that among subjects' variability was not significant (p-value$\leq$0.05) and that the samples were significantly different at 95% confidence level. Monterey Jack was significantly lower in crumbliness than the other samples (p-value$\leq$0.05). The other 3 cheese samples were not significantly different (p-value$\leq$0.05) in crumbliness.

The proposed method had several scope limitations: it does not cover cottage cheese, and for the color attribute, it could not be used for cheese with added colorant(s) and mold-ripened cheese. However, it provided anchors for the attributes of a wide variety of cheeses. The standards were not intended for a specific cheese type, but the attributes' definitions, the anchors, and the way of evaluating them might be adaptable for that purpose.

CONCLUSION

Further study needs to be carried out with more samples, subjects and repetitions to test for each attribute and to look at the variability. A collaborative study might be done to ensure the method's validity. Future study of correlating hand, mouth sensory evaluation and instrumental analysis for cheese texture might be possible. Currently, several studies correlating mouth sensory evaluation and instrumental analysis are available, but there are few publications on correlating hand and mouth sensory evaluation on cheese texture (Drake, M.A et al., 1999).

Even though this study emphasized the attribute of crumbliness, it also evaluated other attributes and look at their correlations with crumbliness. Current results indicate that the proposed attributes, standards and anchors can be used to evaluate sensory characteristics of a wide variety of cheeses. These attributes can describe and distinguish cheeses efficiently. It can be used where hand evaluation is more appropriate, or when many samples need to be tested, since it is less tiring than oral sensory measurement and it avoids olfactory and palate fatigue.

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REFERENCES

Bodyfelt, F.W., Tobias, J., and Trout, G.M. 1988. Sensory Evaluation of Cheese. In: The Sensory Evaluation of Dairy Products. New York: Van Norstrand Reinhold. p300-375.

Brennan, J.G. 1988. Texture Perception and Measurement. In: Piggott, J.R, editor. Sensory Analysis of Foods 2nd Ed. New York: Elsevier Applied Science. p69-101.

Drake, M.A., Gerard, P.D., and Civille, G.V. 1999. Ability of Hand Evaluation versus Mouth Evaluation to Differentiate Texture of Cheese. Journal of Sensory Studies 14(4): 425-441.

Fox, P.F., Guinee, T.P., Cogan, T.M., and McSweeney, P.L.H. 2000. Analytical Methods for Cheese. In: Fundamentals of Cheese Science. Gaithersburg, Maryland: Aspen Publishers, Inc. p523-544.

Hwang, C.H., and Gunasekaran, S. 2001. Measuring crumbliness of some commercial Queso Fresco-type Latin American cheeses. Milchwissenschaft 56(8): 446-450.

Issanchou, S., Schlich, P., and Lesschaeve, I. 1997. Sensory Analysis: Methodological Aspects Relevant to the Study of Cheese. Le Lait: 77(1): 5-12.

Kilcast, D. 1999. Sensory Techniques to Study Food Texture. In: Roshental, A.J., editor. Food Texture: Measurement and Perception. Gaithersburg, Maryland: Aspen Publishers, Inc. p30-64.

Lawless, H.T., and Heymann, H. 1999. Texture Evaluation. In: Sensory Evaluation of Food: Principles and Practices. Gaithersburg, Maryland: Aspen Publishers, Inc. p379-405.

Lee, C.H., Imoto, E.M., and Rha, C. 1978. Evaluation of cheese texture. Journal of Food Science 43(5): 1600-1605.

SPSS. 2000. SPSS, Inc. Chicago, IL.

CHAPTER 2

SENSORY, COMPOSITIONAL AND TEXTURE PROFILE ANALYSIS OF HIGH-PRESSURE TREATED FRESH RENNETED CHEESE – QUESO FRESCO STYLE

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ABSTRACT

Three types of cheese were made: raw cheese (RC), high pressure (HHP) treated raw cheese (HP), and cheese made from HHP treated milk (HPM)). Sensory attributes, compositions, and microstructures were compared. One and eight days storage times were studied. HHP treatment of cheese and milk (400 MPa, 20 min, ambient temperature) were shown to reduce microbial loads. HP and RC had similar microstructure, compositional (p-value$\leq$0.05), and sensory attributes, except color (p-value$\leq$0.05). Microscopy revealed that HP and RC had a distinct protein network, while HPM had a diffuse network. HPM was different from both RC and HP. HPM was the least firm, least crumbly, most sticky and oily. HPM day one was firmer, less oily, less springy than day eight. HPM had higher moisture and yield, due to incorporation of denatured whey proteins, than RC and HP cheese. HHP treatment of cheese might be an alternative way to produce Queso Fresco with acceptable attributes and reduced microbial load.

Keywords: Cheese, Queso Fresco, High Pressure Treatment, Sensory, Texture Profile Analysis

INTRODUCTION

Authentic Queso Fresco is a fresh, soft, white cheese that is traditionally made of raw cows' milk and originates from Latin-American countries. It has a mild, salty flavor with crumbly texture and is particularly popular in the southern and western United States. Crumbliness is an important characteristic of Queso Fresco (Clark et al., 2001).

Due to FDA regulations, commercially available Queso Fresco is made from pasteurized milk. Nevertheless, in 1985, 142 cases of *Listeria monocytogenes* infections were associated with contaminated Queso Fresco in Los Angeles County in California (Linnan et al., 1988). In 1997, home made raw milk Queso Fresco was implicated in a *Salmonella typhimurium* DT104 outbreak in Yakima County, Washington (Bell, Hillers, and Thomas, 1999; and Villar et al., 1999).

Pasteurization is widely used to reduce the microbial load of raw milk, thus, providing safer cheese. Pasteurization of milk is intended to kill 99.999% (10^5 log cycle reduction) of spoilage bacteria and all pathogens. Cheese milk is also pasteurized to kill bacteria that may adversely affect cheese quality. However, pasteurization may damage milk's cheese making properties if the heat treatment is too severe because of excessive whey protein denaturation and their subsequent interactions with kappa caseins, which may cause a defect in cheese texture (Fox et al., 2000a). Milk pasteurization has been shown to make cheese with lower firmness due to higher moisture compared with raw milk cheese (Lau, Barbano, & Rasmussen, 1990). Cheese flavor also develops slower and less intensely in cheese made of pasteurized milk due to the enzyme inactivation as well as lack of contribution of the microorganisms present in raw milk (Fox et al., 2000a).

High hydrostatic pressure processing (HHP) is an alternative method to heat treatment that is gaining popularity. HHP does not use heat, instead, it uses a liquid medium to transfer pressure equally from all directions to the food. Research has shown that HHP of milk reduces the microbial load while causing no or minor changes in nutritional properties, and also flavor compounds, because HHP affects non-covalent bonds (Cheftel, 1992; Tewari, Jayas, & Holley; 1999).

HHP treatment of milk between 150-400 MPa causes partial and irreversible casein micelles fragmentation due to the weakening of electrostatic interaction, and disruption of hydrophobic interactions between sub micelles and casein constituents, thus yielding a smaller micelle size (Balci & Wilbey, 1999). Exposure of hydrophobic regions of milk proteins to the surface increases with increasing exposure time and pressure, indicating considerable protein unfolding, which changes the conformational structure of the proteins, thus altering their functional properties (Johnston, Austin, & Murphy, 1992).

Drake et al. (1997) demonstrated that Cheddar cheese made from high pressure treated milk (345 MPa or 586 MPa for 15 min) would produce cheese with higher moisture, inferior texture, and greater wet weight yield than using pasteurized and raw milk. In contrast, reduced fat cheese made from HHP treated milk was found to have similar composition to raw milk cheese and had improved texture compared with pasteurized milk cheese (Molina et al., 2000). Capellas et al. (2001) studied the effect of HHP on Mato (fresh goat's milk cheese), and determined that the composition of treated cheese was relatively similar to the untreated cheese. Their results showed that differences in water retention capacity, texture, microstructure, and nutrient content between the two cheeses were minor (Capellas et al., 2001).

Messens et al. (2000) studied the effect of HHP treatment (50-400 MPa, 1 hour) on Gouda cheese and found that hydrophobic interactions in the cheese protein network decreased with treatment, and that electrostatic and/or hydrogen bonds might have a stabilizing effect on the network.

There are numerous published studies available on HHP effects on cheese milk. However, there is limited amount of research undertaken on HHP of cheese and its effect on textural attributes. The objective of this study was to investigate whether HHP treated raw cheese or cheese made from HHP treated milk could provide cheese with textural characteristics similar to raw milk fresh cheese. It was beyond the scope of this study to provide microbial inactivation data.

MATERIALS AND METHODS

Cheese making

Queso Fresco cheese was made on a bench-top scale based on a modified commercial recipe from Washington State University (Clark et al., 2001). Three types of cheese were made, namely: control or raw cheese (RC), High Pressure treated cheese (HP), and High Pressure treated milk cheese (HPM) which is cheese made from High pressure treated cheese milk (Figure 1.).

Raw milk was obtained from Oregon State University (OSU) Dairy Barn on the day of cheese making. Approximately 16.0 L raw milk (for RC and HP) or high pressure treated milk (for HPM) was transferred to a water bath (Lab-Line Instruments, Inc. Manufactures and Designers, Melrose Park, Illinois, Catalog # 3010-12) and heated to 32°C. After reaching 32°C (± 1°C), 0.20% (w/w) starter culture (F-DVS R-640 culture, batch# 2147941, CHR Hansen, Milwaukee) was added and incubated for 10-12 minutes (pH: 6.5-6.6). Five percent (w/w) 1:40 dilution rennet (CHYMAX, lot # 76274, CHR Hansen, Milwaukee) was added. The cheese milk was then stirred to ensure proper mixing of rennet and allowed to set. Curd was cut to approximately 1 cm cubes at pH of 6.45 (± 0.02) and allowed to heal for five minutes. Heat was increased slowly to 40°C (1°C increase per 5 minutes) while stirring slowly and continuously to minimize curd damage. Temperature was maintained at 40°C and stirred for an additional 20 minutes carefully to minimize curd damage. Salt 1.8% ((w/w)) was weighed and divided into three parts. About 6.0 kg ± 0.1 kg whey was drained (whey pH was 5.7-5.8), and first application of salt (99 g) was added and stirred for 3-5 minutes. Approximately 3.7 kg ± 0.1 kg whey was drained, and the second application of salt (99 g) was added and stirred for 3-5 minutes. About 4.0 kg ± 0.1 kg whey was drained and a third application of salt (99 g) was added and stirred for 3-5 minutes. The reminder of the free whey was drained and the curd was air dried in the vat for 15 minutes. Curd was wrapped in cheese cloth and pressed (18.0-18.3 grams per cm^2) at room temperature for about two hours. If raw milk was used, the cheese was divided into two parts (1 for RC and the other for HP) and vacuum packed. The cheese for HP was then subjected to HHP treatment. Cheeses were stored at refrigeration (4°C) temperature until sensory analysis, and at -23°C for chemical analysis (Figure 1). Each type of cheese was made in triplicate on three separate days.

High hydrostatic pressure (HHP) treatment

Samples (milk or cheese) were vacuum packed and put into polyethylene plastic bags (KAPAK, Minneapolis, MN) prior to HHP treatment. HHP treatment of 400 MPa, 20 minutes, at ambient (approximately 20°C) temperature was employed (Engineering Pressure System Inc., 22 L capacity, Andover, MA). The time needed to reach pressure was about 5 to 9 minutes, and pressure was released instantly after treatment.

Compositional analysis

All chemicals used were analytical grade and obtained from one of the following sources: J. T. Baker, Fischer Scientific or VWR. Triplicates were done on each analysis. pH (pH meter AR 25 Accumet Research Dual Channel, pH/Ion meter, Fisher Scientific), protein, fat, and moisture contents were analyzed. Micro Kjeldahl was used to measure the amount of protein (%protein=6.38x%N) (Richardson, 1985). The distillation unit used was a Rapid Still II Labconco. The Babcock method was used to measure the fat content (Richardson, 1985). Babcock centrifuge used was Cherry-Burrell Babcock centrifuge model TD-24H. Moisture content was measured by forced-draft oven (Fisher isotemp forced draft oven) at 102 ± 2 °C until a constant weight was reached (Capellas et al., 2001). An Osterizer-10 cycle blend pulsematic blender was used to homogenize the samples.

Sensory analysis

Descriptive sensory analysis with ten trained panelists assessed the samples by hand evaluation in an individual booth with natural light. A sixteen point intensity scale was used where 0=no attribute evident, 15=very extreme evidence of the attribute. The panelists identified 15 attributes that were grouped into five categories: appearance before breaking, texture before breaking, texture during breaking, residuals left after breaking, and appearance after breaking were tested. Each panelist evaluated the samples in duplicate and in a randomly assigned presentation order. Samples and standards were cut at refrigeration temperature and equilibrated to room temperature at least one hour prior to presentation, and placed into an individual cup with lid and labeled with a three digit random numbers. Sample and standard size were 1.5 x 1.5 x 1.5 cm cubes. Standards were presented on plates wrapped in saran plastic wrap. Crumbliness was defined, by panelists' consensus, as: the ease of the sample to break apart and crumble during manipulation using the thumb and two fingers for five times.

Texture Profile Analysis (TPA) and compression test

Sample size for TPA and compression test was 1.5 x 1.5 x 1.5 cm cubes. The samples were cut at refrigeration temperature and equilibrated to room temperature prior to analysis. Texture analyzer (TA-XT2iHR, Stable Micro Systems, Carry, NY) with Texture Expert software version 07.15H and load cell of 5 kg was used for TPA and compression analysis. Conditions for TPA analysis were 25% compression, 0.4 mm/s speed, 5 seconds time interval between first and second compression, while that of compression test utilized 80% compression, and 0.4 mm/s speed. The first detected peak of the compression test would indicate sample fracturability, while the maximum force peak indicated firmness.

Microbiological analysis

All microbiological analyses were plated on 3M petrifilm (3M, St. Paul, MN) and incubated based on the procedure in Standard Methods for the Examination of Dairy Products (Richardson, 1985). Aerobic Plate Count (3M Aerobic Count Plate), Coliform Count (*Escherichia coli*/Coliform Count Plate), and Yeast & Mold Count (Yeast and Mold Count Plate) were done on the milk, and whey samples. Only Coliform and Yeast & Mold Counts were done on the cheese samples.

Protein microstructure

Protein microstructure was observed by Acid Fuschin (Sigma #F-8129, lot# 111F-0047) protein staining and light microscope (Clark, 1973). Solvent used was deionized distilled water. Dye (Acid Fuschin) solution, 0.1% (w/v) aqueous solution, was prepared and stored at room temperature and protected from light.

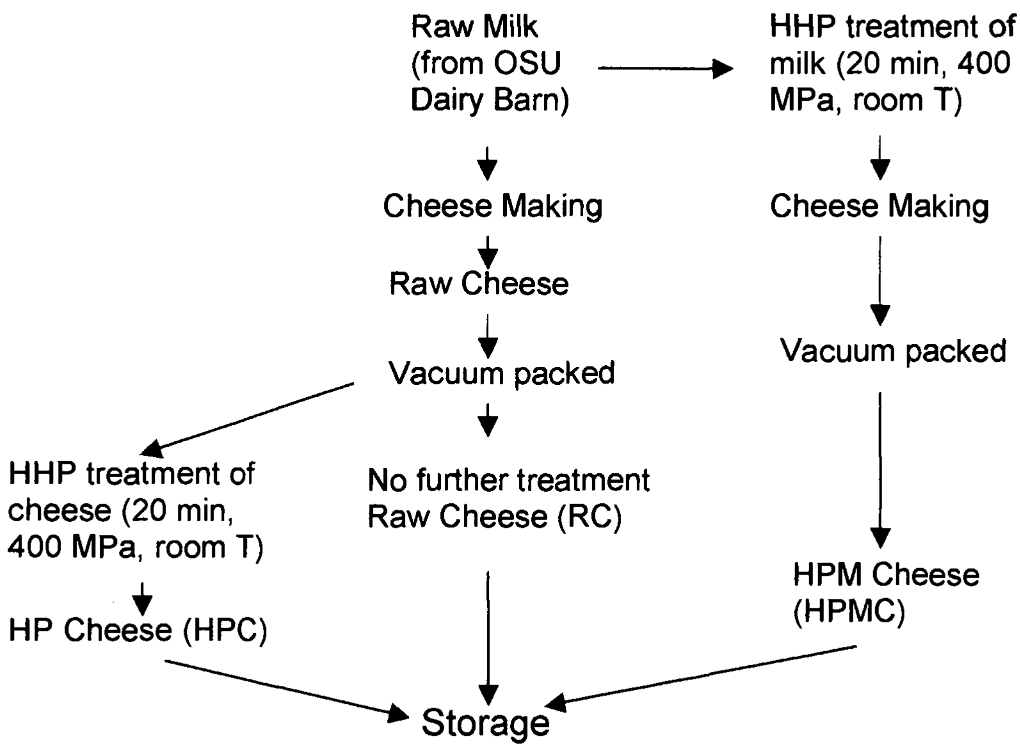
Gel electrophoresis

All equipment (EC 105, E-C Apparatus Corporation), sample buffers, molecular weight standards, running buffers, 10-20% Tris HCl-30μL load size PAGE, staining and destaining solutions were obtained from BIORAD (Biorad, Hercules, CA). Sample preparation was carried out as follows: cheese, milk, and whey samples were diluted 1:9, 1:4, 1:1, respectively with 10 mM phosphate buffer (pH 6.84) and then centrifuged for 30 minutes at 14000 rpm. The supernatant was used for gel electrophoresis. For native gel electrophoresis, sample was diluted 1:2 with native sample buffer, except for raw whey (1:4) to make the protein content approximately similar to whey from HPM, prior to loading into the wells. For SDS PAGE, samples were diluted 1:3 with Laemli sample buffer (beta mercapthoethanol added), except for raw whey (1:6) to make the protein content close to that of whey from HPM. Diluted samples and molecular weight standards were diluted 1:20, then samples were heated at 95°C for 10 minutes, cooled and then loaded to the wells.

Statistical analysis

Tukey-HSD, Analysis of Variance (ANOVA), and Generalized Procrustes Analysis (GPA) were used to analyze the data (SPSS v. 10.1, SPSS Inc., Chicago, IL). P-value$\leq$0.05 was used throughout the analysis. ANOVA was applied to determine if any of the samples was different for each attribute tested. GPA was employed to determine which attributes were important in describing and differentiating samples, while combining all the data and treating them independently. Sample comparison was determined by Tukey-HSD to include all possible sample comparisons for each attribute.

Figure 2.1 Flow diagram of three different cheese types made (RC, HPC, and HPMC).



RESULTS AND DISCUSSIONS

Sensory analysis

Table 1 displays the means and standard deviations of each attribute tested by descriptive panel. HPM was the least crumbly, while RC was the most crumbly among the cheeses. Firmness of RC and HP were similar and higher than HPM. Firmness and crumbliness showed similar trends among the cheeses, which indicated a possible positive correlation. Moistness appearance, wet and oil residual of HP were similar to RC, while HPM exhibited higher scores. Stickiness and sticky residual were highest for HPM, and the least for RC. However, HPM oiliness was the highest among the cheeses. Cohesiveness and piece size of HPM were highest, followed by HP and RC, while the scores for number of pieces observed after manipulation, in decreasing order, were RC, HP, and HPM. The more cohesive the cheese was, the bigger the piece size obtained after manipulation, and the less number of pieces obtained. Overall, HP had more attributes similar to RC, while HPM was perceived as the most different among the samples.

RC did not exhibit any changes in attributes tested for the storage time studied. HP color day one was perceived as more yellow than day 8. No storage time effect was observed in moistness appearance, wet and oily residual characteristics of HP and RC. Sticky and sticky residuals of HP increased significantly ($p\text{-value} \leq 0.05$) during storage. Firmness, surface irregularity, and oiliness appearance changed significantly during HPM storage time.

TPA and compression test

TPA results (Table 2) showed significant ($p\text{-value} \leq 0.05$) differences among the three cheese types studied in firmness, gumminess, and chewiness. HPM exhibited the lowest firmness, which corresponded to the sensory results. HPM also had the lowest gumminess, and chewiness. Springiness was not found to be different among samples. Cohesiveness of RC, HP and HPM day 8 were similar, while HPM day 1 had the lowest value. Resilience was not found to be significantly different among samples. There was no storage time effect on firmness, gumminess, and chewiness of RC and HPM, however a decrease in these attributes was observed in HP.

Distance, % compression, and force of the first peak, obtained from compression test, are indications of fracturability (Table 3). Highest distance and % compression were observed for HPM, suggesting that HPM was the least crumbly among the samples. Force at the first peak for HPM was lower than both RC and HP, but the first peak observed for HPM was also the maximum peak, thus demonstrating that HPM did not fracture at all. HPM day 8 had the lowest force at the first peak detected, indicating that it was the least firm as confirmed by the sensory results. Distance, % compression, and force of the first peak of RC day 1, RC day 8, HP day 1, and HP day 8 were not significantly different (at 95% confidence level). Once again this correlates with sensory results which found few differences between RC and HP. The results indicated that sensory and instrumental analyses were equally good in describing and differentiating the cheeses.

Composition

HHP treatment on milk did not change the pH, moisture, protein and fat content of milk (Table 4). However, lower amounts of protein and fat were obtained in whey from HPM cheese making, suggesting that more fat and protein were incorporated into HPM cheese. Table 5 showed that protein content of HPM cheese was higher than RC. Fat content of HPM cheese was lower than RC, a possible explanation for this was that the fat droplets were not tightly bound in the cheese matrix, because of the lack of protein network, and were lost during sample preparation. The moisture content of HPM was the highest among the samples, suggesting an increased in water binding capacity, possibly due to incorporation of

Table 2.1 Mean differences in sensory (hand panel) attributes among RC, HP and HPM cheeses stored for 1 and 8 days.

Attributes	RC day 1	RC day 8	HP cheese day 1	HP cheese day 8	HPM cheese day 1	HPM cheese day 8
color	6.0 ^{a,b} (0.2)	6.1 ^{a,b} (0.2)	7.0 ^{c} (0.2)	6.6 ^{b,c} (0.2)	5.6 ^{a} (0.2)	5.8 ^{a} (0.2)
moistness	4.8 ^{a} (0.2)	5.1 ^{a} (0.2)	4.5 ^{a} (0.2)	4.7 ^{a} (0.2)	8.7 ^{b} (0.3)	9.0 ^{b} (0.5)
oiliness	6.6 ^{a} (0.3)	6.8 ^{a} (0.2)	7.7 ^{a,b} (0.3)	7.0 ^{a} (0.2)	8.4 ^{b} (0.3)	9.7 ^{c} (0.5)
irregularity	7.0 ^{a} (0.4)	6.0 ^{a,b} (0.4)	4.0 ^{c} (0.2)	5.4 ^{b,c} (0.3)	8.7 ^{d} (0.4)	10.6 ^{e} (0.5)
firmness	6.2 ^{a} (0.4)	6.1 ^{a} (0.2)	6.0 ^{a} (0.2)	6.1 ^{a} (0.3)	3.0 ^{b} (0.2)	1.9 ^{c} (0.2)
springiness	6.0 ^{a,b} (0.2)	5.5 ^{a} (0.2)	6.5 ^{b} (0.2)	6.0 ^{a,b} (0.2)	1.7 ^{c} (0.2)	0.8 ^{d} (0.2)
crumbliness	7.5 ^{a} (0.3)	6.9 ^{a} (0.3)	5.7 ^{b} (0.2)	5.4 ^{b} (0.3)	1.3 ^{c} (0.2)	0.5 ^{c} (0.1)
stickiness	6.5 ^{a} (0.3)	7.1 ^{a} (0.4)	7.5 ^{a} (0.3)	8.8 ^{b} (0.3)	13.3 ^{c} (0.2)	14.3 ^{c} (0.1)
cohesiveness	6.2 ^{a} (0.3)	7.0 ^{a,b} (0.3)	8.1 ^{b,c} (0.3)	8.6 ^{c} (0.3)	12.7 ^{d} (0.4)	13.7 ^{d} (0.2)
sticky residue	5.9 ^{a} (0.2)	6.4 ^{a} (0.3)	6.8 ^{a} (0.3)	8.0 ^{b} (0.4)	13.3 ^{c} (0.2)	14.2 ^{c} (0.1)
wet residual	5.4 ^{a} (0.3)	4.8 ^{a} (0.2)	5.1 ^{a} (0.3)	5.6 ^{a} (0.3)	8.9 ^{b} (0.4)	9.7 ^{b} (0.5)
oil residual	7.7 ^{a} (0.4)	7.9 ^{a} (0.3)	8.6 ^{a} (0.4)	8.9 ^{a} (0.3)	11.8 ^{b} (0.3)	12.2 ^{b} (0.4)
piece size	7.6 ^{a} (0.2)	7.7 ^{a} (0.2)	8.8 ^{a,b} (0.3)	9.2 ^{b} (0.4)	12.4 ^{c} (0.4)	12.6 ^{c} (0.6)
Number of pieces	5.66 ^{a,b} (0.24)	5.8 ^{a} (0.3)	4.8 ^{b} (0.2)	4.98 ^{a,b} (0.29)	1.8 ^{c} (0.2)	1.1 ^{c} (0.2)
Piece size uniformity	10.4 ^{a,b} (0.3)	10.4 ^{a,b} (0.4)	10.0 ^{a,b} (0.4)	9.8 ^{a} (0.4)	11.6 ^{a,b} (0.5)	11.7 ^{b} (0.7)

$^{a, b, c, d, e}$ the same letters indicate the mean difference is not significantly different at p-value$\leq$0.05.

Values are reported as mean (SD) of triplicate samples analyzed in duplicates.

Table 2.2 Mean differences of Texture Profile Analysis (TPA) properties of RC, HP, and HPM cheeses stored for 1 and 8 days.

TPA	RC day 1	RC day 8	HP cheese day 1	HP cheese day 8	HPM cheese day 1	HPM cheese day 8
Firmness	573 [a] (35)	659 [a,b] (10)	737[b] (19)	417 [c] (28)	73 [d] (12)	43 [d] (10)
Springiness	0.80 [a] (0.01)	0.78 [a] (0.01)	0.78 [a] (0.01)	0.77 [a] (0.01)	0.92 [a] (0.12)	0.75 [a] (0.03)
Cohesive-ness	0.73 [a] (0.004)	0.72 [a] (0.004)	0.73 [a] (0.003)	0.71 [a] (0.01)	0.63 [b] (0.02)	0.70 [a] (0.03)
Gumminess	419 [a] (28)	471 [a] (5)	539 [b] (14)	315 [c] (14)	46 [d] (8)	30 [d] (7)
Chewiness	336 [a] (23)	367.811 [a] (4.421)	421 [b] (9)	239 [c] (8)	42 [d] (8)	22 [d] (5)
Resilience	0.36 [a] (0.004)	0.35 [a] (0.01)	0.35 [a] (0.003)	0.33 [a,b] (0.01)	0.26 [a,b] (0.04)	0.24 [b] (0.04)

[a, b, c, d] the same letters indicate the mean difference is not significantly different at p-value≤0.05.
Values are reported as mean (SD) of triplicate samples and five TPA measurements.

Table 2.3 Mean differences in compression (80% compression) instrumental test of RC, HP, and HPM cheeses stored for 1 and 8 days.

Properties	RC day 1	RC day 8	HP cheese day 1	HP cheese day 8	HPM cheese day 1	HPM cheese day 8
Distance [1] (mm)	12.6 [a] (0.8)	12.5 [a] (0.3)	11.0 [a] (0.4)	12.0 [a] (0.4)	15.4 [b] (0.4)	15.7 [b] (0.1)
% compression [2]	63.2 [a] (3.8)	62.6 [a] (1.5)	55.0 [a] (1.6)	59.9 [a] (1.9)	77.1 [b] (1.8)	78.6 [b] (0.6)
Force at first peak (g)	3143 [a] (4)	3144 [a] (2)	3147 [a] (2)	3146 [a] (2)	2696 [b] (194)	682 [c] (54)

[1] Distance traveled (measured from the top of sample cube) by the probe (during compression) when the first peak was detected

[2] % compression of sample cube by probe when the first peak was detected. Calculated as distance (mm)/20 mm*100. 100% compression = 20.0 mm in distance (measured from top of cube) traveled by the probe.

[a, b, c] the same letters indicate the mean difference is not significantly different at p-value≤0.05.

Values are reported as mean (SD) of triplicate samples and five measurements.

Table 2.4 Composition analysis of raw and HHP treated milk and whey from RC and HPM cheese making.

	RAW MILK	HP MILK	RAW WHEY	HP WHEY
pH	6.74 (0.01) [a]	6.70 (0.02)[a]	5.42 (0.10)[b]	5.71 (0.00) [c]
% moisture	86.9 (0.2)[a]	87.0 (0.2)[a]	93.2 (0.1)[b]	93.4 (0.1)[b]
% solids[1]	13.1 (0.2)[a]	13.0 (0.2)[a]	6.8 (0.1)[b]	6.6 (0.1)[b]
% fat (as is)	3.5 (0.1)[a]	3.6 (0.2)[a]	1.4 (0.1)[b]	1.0 (0.1)[c]
% fat (dry basis)[2]	26.9 (0.8)[a]	28.0 (1.2)[a]	20.4 (1.2)[b]	15.7 (1.2)[c]
% protein (as is)	2.73 (0.08)[a]	2.60 (0.07)[a]	0.48 (0.03)[b]	0.26 (0.03)[c]
% protein (dry basis)[2]	20.9 (0.7)[a]	19.9 (1.2)[a]	7.1 (0.4)[b]	4.0 (0.5)[c]

[1] calculated as 100%-%moisture

[2] calculated as (as is/%solids)*100

[a, b, c] the same letters indicate the mean difference is not significantly different at p-value≤0.05

Values are reported as mean (SD) of triplicate samples and triplicate measurements.

Table 2.5 Composition, pH, and yield of RC, HP, and HPM cheeses stored for 1 and 8 days.

COMPOSITION	Raw cheese day 1	Raw cheese day 8	HP cheese day 1	HP cheese day 8	HPM cheese day 1	HPM cheese day 8
pH	5.16 (0.03) [a]	5.16 (0.02) [a]	5.22 (0.01) [b]	5.20 (0.01) [b]	5.13 (0.02) [a]	5.10 (0.04) [a]
% moisture	45.7 (1.4) [a]	45.1 (0.6) [a]	45.4 (0.4) [a]	45.1 (0.6) [a]	55.7 (0.6) [b]	55.0 (0.7) [b]
% solids[1]	54.3 (1.4) [a]	54.9 (0.6) [a]	54.6 (0.4) [a]	54.9 (0.6) [a]	44.3 (0.6) [b]	45.0 (0.7) [b]
% fat (as is)	28.4 (0.6) [a]	NA	26.8 (1.2) [a]	NA	21.4 (0.5) [b]	NA
% fat (dry basis)[2]	52.4 (1.7) [a]	NA	49.2 (2.3) [a, b]	NA	48.3 (1.3) [b]	NA
% protein (as is)	20.7 (0.6) [a]	NA	23.4 (1.2) [a]	NA	20.4 (1.5) [a]	NA
% protein (dry basis)[2]	38.2 (1.4) [a]	NA	42.8 (2.3) [a, b]	NA	46.0 (3.3) [b]	NA
% yield (as is)	11.9 (0.7) [a]	NA	NA	NA	17.4 (0.5) [b]	NA
% yield (dry basis) [2,3]	21.9 (1.5) [a]	NA	NA	NA	39.2 (1.3) [b]	NA

[1] calculated as 100%-%moisture
[2] calculated as (as is/%solids)*100
[3] calculated as 100*(weight of cheese / (weight of milk + culture + salt))
[a, b] the same letters indicate the mean difference is not significantly different at p-value$\leq$0.05
Values are reported as mean (SD) of triplicate samples and triplicate measurements.
NA: Not Available.

denatured whey proteins into the cheese. Another explanation possible is that HHP treatment of milk reduced the ability of casein micelles and fat globules to agglomerate closely, thus allowing moisture to be trapped and retained in the cheese (O'Reilly et al., 2001). Percent yield of HPM was higher than RC due to incorporation of moisture and protein. It is well known that HHP denatures whey proteins, exposing hydrophobic and free –SH groups, hence making it possible to form hydrophobic and/or disulfide bonds with other protein(s) or self aggregation, which might explain the incorporation of the whey proteins into the cheese (Balci, and Wilbey, 1999). HP composition was not significantly different from RC (p-value$\leq$0.05). pH of HP was higher than RC and HPM, most likely due to pressure induced paracasein network dissociation, exposing the alkaline groups (Messens et al., 1999).

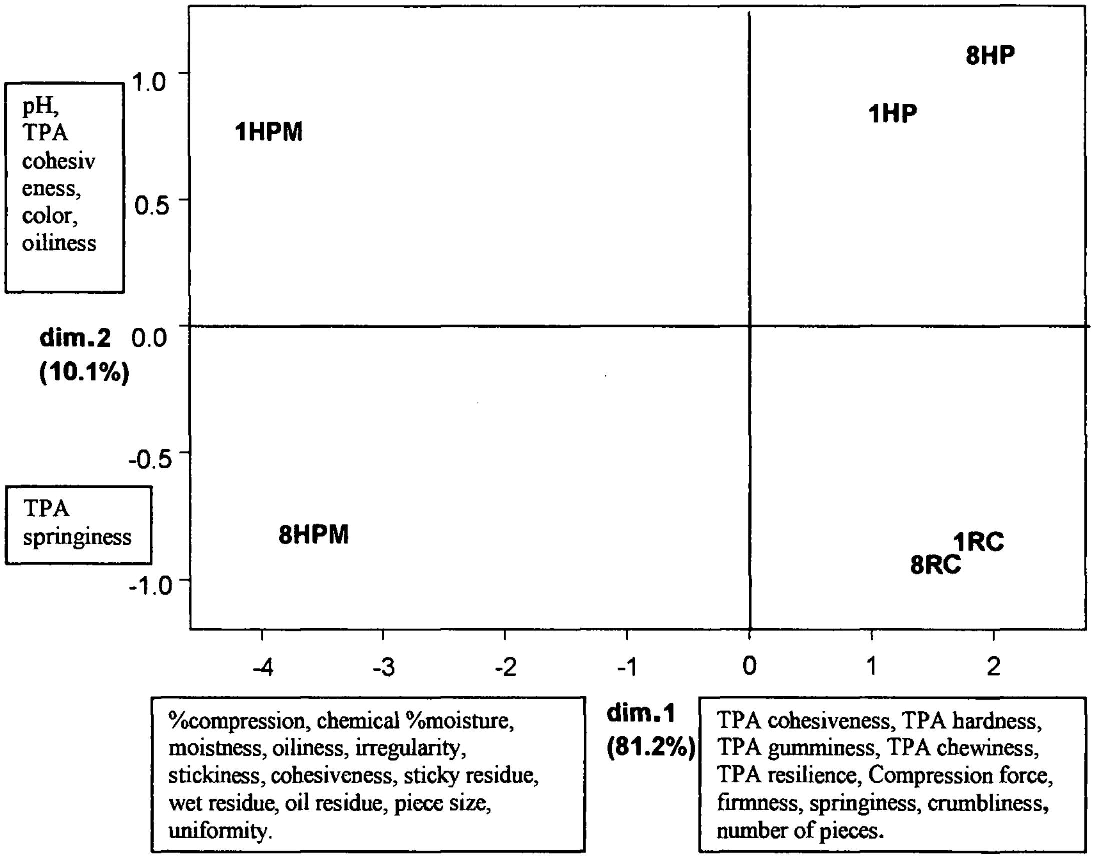
Generalized Procrustes Analysis (GPA)

GPA is the statistical method used to summarize the results obtained by instrumental, sensory and chemical data (Figure 2). GPA grouped the samples into four groups, namely: RC, HP, HPM day 1, and HPM day 8. HP cheese had similar characteristics as RC, except pH and color. HPM cheese was different from HP and RC in all properties, except TPA springiness and cohesiveness. HPM day 1 and 8 were different in oiliness appearance. In conclusion, HP was more similar to RC in most of the attributes (including crumbliness), while HPM cheese was different.

Microbial analysis

HHP treatment of both milk and cheese caused reduction in microbial load. HPM and HP had lower microbial load than RC. HHP treatment of milk caused reductions in aerobic plate count (96%), coliform (97%) and yeast & mold count (87%), while HHP treatment of cheese caused reductions of coliform (90%), and yeast & mold counts (67%). It appears that HHP treatment of cheese or cheese

Figure 2.2 GPA Plot indicating the grouping of samples based on dimension 1 and 2 extracted, with the attributes associated. X-axis is dimension 1 (explaining 81.2% variation), Y-axis is dimension 2 (explaining 10.1% variation). 1RC= RC day 1, 8RC= RC day 8, 1HP= HP cheese day 1, 8HP=HP cheese day 8, 1HPM= HPM cheese day 1, 8HPM=HPM cheese day 8.



milk at 400 MPa for 20 minutes at ambient temperature has approximately the same effect on microbial destruction. However, additional research is required to confirm these results.

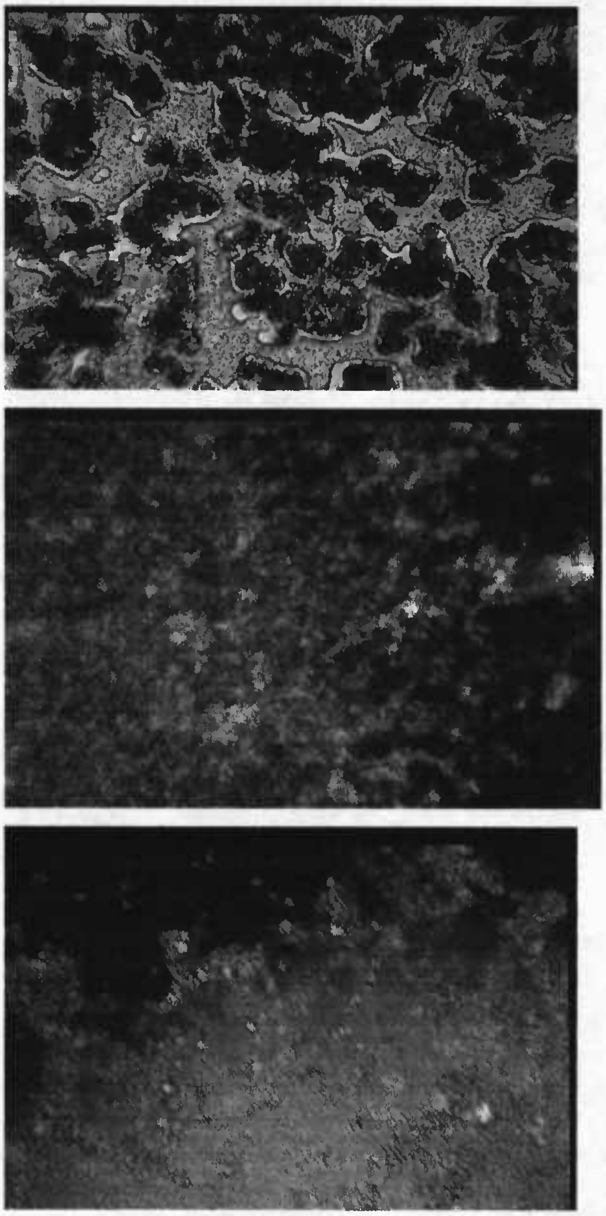
Cheese microstructure

RC showed a well-defined protein network and protein encapsulated fat globules (Figure 3). The HP cheese still contained protein encapsulated fat globules, even though the protein network (casein network) was less distinct than in RC. HPM cheese had lost its protein network and little or no protein encapsulated fat globules were present. Free fat globules and moisture droplets were observed, which explained why weak and oily texture was obtained. Due to the lack of protein network, more oil was released during storage, thus HPM day 8 was more oily than day 1, as perceived by the panelists. This finding is consistent with O'Reilley et al. (2001) who have reported that large pools of fat globules were observed in cheese made from pressurized goats' milk. HHP treatment of milk caused protein denaturation and altered milk cheese making properties, hence causing textural changes, as reported in previous research (Johnston, Austin, and Murphy, 1992; and Drake et al, 1997).

Gel electrophoresis

Native gel electrophoresis (Figure 4) showed that both raw milk and whey had more of the lower molecular weight proteins present when compared with HHP treated samples. HHP of cheese milk caused protein agglomeration in the cheese as shown by the presence of darker bands of higher molecular weight proteins in HPM. HHP treatment of raw cheese did not seem to have significant effect on the protein agglomeration in the cheese as indicated by the similar protein bands profile and intensity between RC and HPC. SDS-PAGE (Figure 5) was done to show subunits of the protein agglomeration. Based on SDS-PAGE, there was no obvious difference among the three cheeses. However, HHP

Figure 2.3 Light microscopy pictures (250x) of protein network (by Acid Fuschin protein staining) in cheese (a) RC, (b) HP, and (c) HPM.



(a)

(b)

(c)

Figure 2.4 Native PAGE of cheese, milk and whey samples. Well 1: standard MW, well 3: RC, well 4: HP cheese, well 5: HPM cheese, well 6: raw milk, well 7: HP milk, well 8: raw whey, and well 9: HP whey.

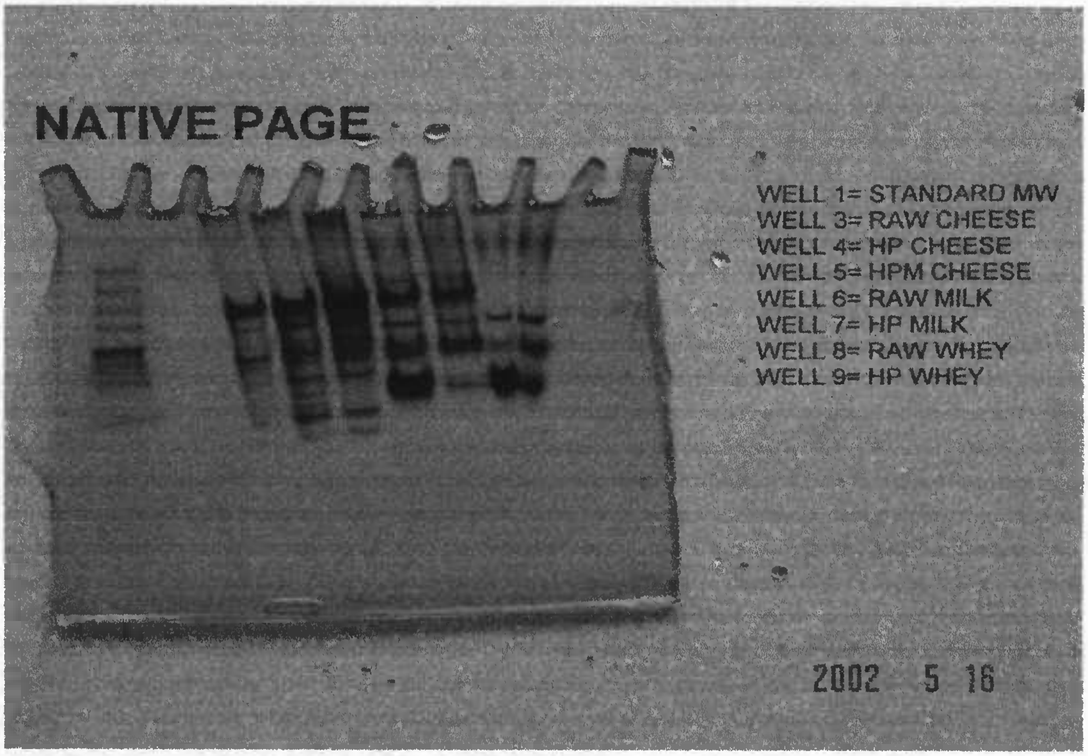
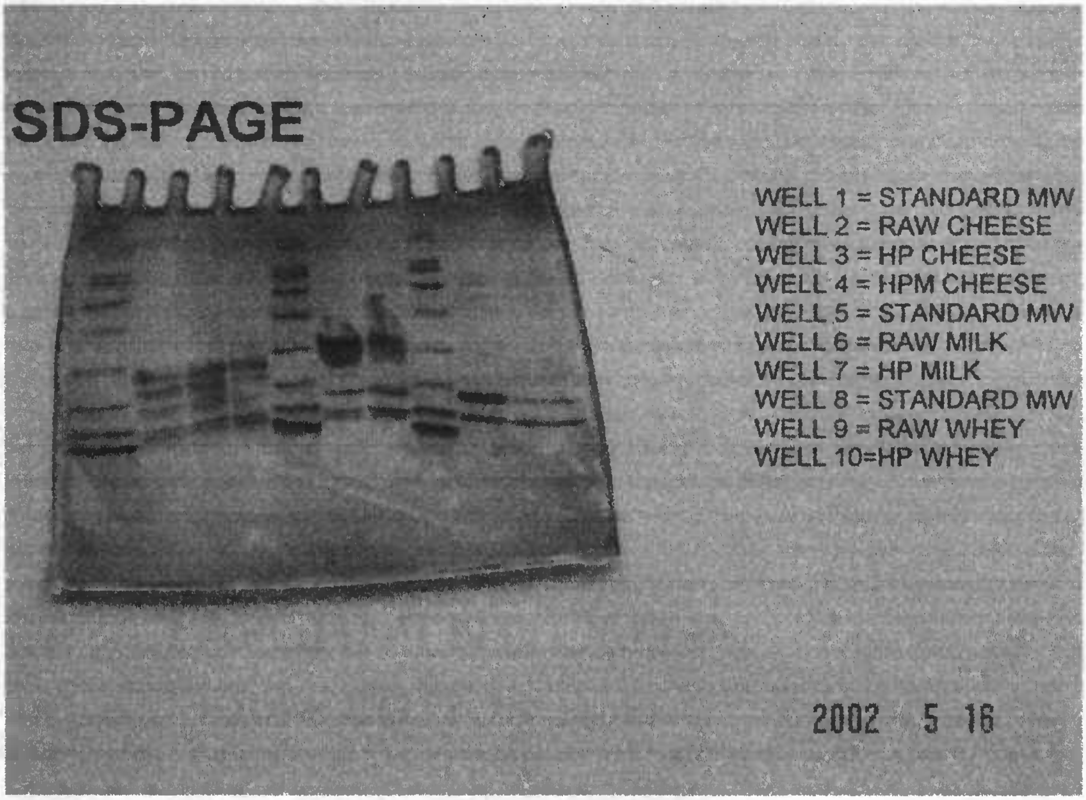


Figure 2.5 SDS-PAGE of cheese, milk, and whey samples. Lanes 1, 5, and 8: MW standards, lane 2: RC, lane 3: HP cheese, lane 4: HPM cheese, lane 6: raw milk, lane 7: HP milk, lane 9: raw whey, and lane 10: HP whey.



treatment of milk caused disruption of casein micelles as indicated by less intensity of casein bands in the HHP milk sample, as reported previously by Needs at al. (2000). Hydrolysis and/or disintegration of casein micelles have been shown to promote decreases in firmness, which explained why HPM cheese had the softest texture among the samples (Fox et al., 2000b).

Whey from HPM cheese showed less dark band of β-Lg than raw whey. It seemed likely that more whey proteins, particularly β-Lg, because of its pressure sensitive nature, were incorporated into the cheese made from HHP treated milk. The incorporation of denatured β-Lg into HPM cheese might be the cause of the higher moisture content due to the increase of water binding capacity, and thus resulted in the softer texture of HPM cheese (Lopez-Fandino et al., 1996; and Drake et al., 1997). The increase in moisture and the incorporation of more whey proteins also caused the yield and the protein content to increase.

CONCLUSION

HHP treatment of both cheese and milk caused reduction in microbial load. To measure the significance of the reduction, a different study should be designed utilizing bacteria inoculation. HP cheese had similar sensory attributes and microstructure as RC, although the color of HP cheese was more yellow than RC. HPM cheese was different from HP and RC in almost all of the attributes tested. HHP treatment of milk appears to have caused whey proteins denaturation and a disruption in the protein-fat network, possibly causing the high moisture content, weak texture, as well as high oiliness and stickiness. HHP treatment caused protein aggregation and increased incorporation of whey proteins into the cheese. HHP treatment of cheese did not impart significant textural and compositional changes, hence, HHP treatment of raw cheese can be an alternative method to provide Queso Fresco with lower microbial load, acceptable sensory attributes and similar composition to traditional Queso Fresco.

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REFERENCES

Allogio, V., Caponio, F., Pasqualone, A., & Gomes, T. (2000). Effect of heat treatment on the rennet clotting time of goat and cow milk. Food Chemistry, 70, 51-55.

Alpas, H., Kalchayanand, N., Bozoglu, F., Sikes, A., Dunne, C. P., and Ray, B. (1999). Variation in resistance to hydrostatic pressure among strains of food-borne pathogens. Applied and Environmental Microbiology, 65, 4248-4251.

Arias, M., Lopez-Fandino, R., and Olano, A. 2000. Influence of pH on the effects of high pressure on milk proteins. Milchwissenschaft, 55, 191-194.

Balci, A. T., & Wilbey, R. A. (1999). High pressure processing of milk- the first 100 years in the development of a new technology. International Journal of Dairy Technology, 52, 149-155.

Bell, R. A., Hillers, V. N., and Thomas, T. A. (1999). The Abuela Project: safe cheese workshops to reduce the incidence of Salmonella typhimurium from consumption of raw-milk fresh cheese. American Journal of Public Health, 89, 1421-1424.

Benito, A., Ventoura, G., Casadei, M., Robinson, T., and Mackey, B. (1999). Variation in resistance of natural isolates of Escherichia coli O157 to high hydrostatic pressure, mild stresses. Applied and Environmental Microbiology, 65, 1564-1569.

Birkkjaer, H. E., Soerensen, E. J., Joergensen, J., and Sigersted, E. (1961). The influence of the cheese making technique upon the quality of cheese. In Danish Government Research Institute for Dairy Industry, Hilleroed, Report no 128.

Bourne, M. C. (1976). Interpretation of force curves from instrumental texture measurements. In J.M. deMan, P.W. Voisey, V. F. Rasper, and D. W. Stanley, Eds. Rheology and Texture in Food Quality (pp 244-274). Westport, CT: Avi Publishing Co.

Buffa, M.N., Trujillo, A. J., Pavia, M., & Guamis, B. (2001a). Changes in textural, microstructural, and color characteristics during ripening of cheeses made from raw, pasteurized or high-pressure-treated goats' milk. International Dairy Journal, 11, 927-934.

Buffa, M. N., Guamis. B., Pavia., M., & Trujillo, A. J. (2001b). Lipolysis in cheese made from raw, pasteurized or high-pressure-treated goats' milk. International Dairy Journal, 11, 175-179.

Capella, M., Mor-Mur, M., Sendra, E., & Guamis, B. (2001). Effect of high-pressure processing on physico-chemical characteristics of fresh goats' milk cheese (Mato). International Dairy Journal, 11, 165-173.

Cheftel, J. C. (1992). Effects of high hydrostatic pressure on food constituents: an overview. High Pressure and Biotechnology, 224, 195-209.

Cheftel, J. C. (1995). Review: High-pressure, microbial inactivation and food preservation. Food Science and Technology International, 1, 75-90.

Clark, G. (1973). Staining Procedures Used by the Biological Stain Commission. Baltimore: Williams & Wilkins.

Clark, S., Warner, H., & Luedecke, L. (2001). Acceptability of Queso Fresco cheese by traditional and nontraditional consumers. Food Science Technology Int, 7, 165-170.

Corredig, M., and Dalgleish, D. G. (1996). The binding of α-lactalbumin and β-lactoglubulin to casein micelles in milk treated by different heating systems. Milchwissenschaft, 51, 123-126.

Dijksterhuis, G. B. (1997). Interpreting generalized procrustes analysis "Analysis of Variance" tables. In Multivariate Data Analysis in Sensory & Consumer Science (pp77-215). Connecticut: Food & Nutrition Press, Inc.

Drake, M.A., Harrison, S.L., Asplund, M., Barbosa-Canovas, G., and Swanson. B.G. (1997). High pressure treatment of milk and effects on microbiological and sensory quality of Cheddar cheese. Journal of Food Science 62(4): 843-845.

Drake, M.A., Gerard, P.D., and Civille, G.V. (1999). Ability of hand evaluation versus mouth evaluation to differentiate texture of cheese. Journal of Sensory Studies 14(4): 425-441.

Earnshaw, R. G. (1995). Kinetics of high pressure inactivation of microorganisms. In D. A. Ledward, D. E. Johnston, R. G. Earnshaw, and A. M. P. Hasting (Eds). High pressure processing of foods (pp 37-46). Loughborough: Notthingham University Press.

Ellenberg, L., and Hoover, D. G. (1999). Injury and survival of Aeromonas hydrophila 7965 and Yersenia enterocolitica 9610 from high hydrostatic pressure. Journal of Food Safety, 19, 263-276.

Felipe, X., Capellas, M., and Law, A. J. R. (1997). Comparison of the effects of high-pressure treatments and heat pasteurization on the whey proteins in goat's milk. Journal of Agriculture and Food Chemistry, 45, 627-631.

Fox, P. F., Guinee, T. P., Cogan, T. M., & McSweeney, P. L. H. (2000a). Bacteriology of cheese milk. In Fundamentals of Cheese Science (pp 47-50). Gaithersburg, Maryland: Aspen Publishers Inc.

Fox, P. F., Guinee, T. P., Cogan, T. M., & McSweeney, P. L. H. (2000b). Cheese rheology and texture. In Fundamentals of Cheese Science (pp 305-340). Gaithersburg, Maryland: Aspen Publishers Inc.

Garcia-Graells, C., Valckx, C., and Michiels, C. W. (2000). Inactivation of Escherichia coli and Listeria innocua in milk by combined treatment with high hydrostatic pressure and the lactoperoxidase system. Applied and Environmental Microbiology, 66, 4173-4179.

Garcia-Risco, M. R., Olano, A., Ramos, M., and Lopez-Fandino, R. (2000). Micellar changes induced by high pressure, influence in the proteolytic activity and organoleptic properties of milk. Journal of Dairy Science, 83, 2184-2189.

Gervilla, R., Ferragut, V., and Guamis, B. (2000). High pressure inactivation of microorganisms inoculated into ovine milk of different fat contents. Journal of Dairy Science, 83, 674-682.

Gervilla, R., Sendra, E., Farragut, V., and Guamis, B. (1999). Sensitivity of Staphylococcus aureus and Lactobacillus helveticus in ovine milk subjected to high pressure. Journal of Dairy Science, 82, 1099-1107.

Heinz, V., and Knorr, D. (1996). High pressure inactivation kinetics of Bacillus subtilis cells by a three-state-model considering distributed resistance mechanisms. Food Biotechnology, 10, 149-161.

Hoover, D. G., Metrick, C., Papineau, A. M., Farkas, D. F., and Knorr, D. (1989). Biological effects of high hydrostatic pressure on food microorganisms. Food Technology, 99-107.

Johnston, D. E., Austin, B. A., & Murphy, R. J. (1992). Effects of high hydrostatic pressure on milk. Milchwissenschaft, 47, 760-763.

Kalchayanand, N., Sikes, A., Dunne, C. P., and Ray, B. (1998). Interaction of hydrostatic pressure, time and temperature of pressurization and Pediocin AcH on inactivation of foodborne bacteria. Journal of Food Protection, 61, 425-431.

Kheadr, E. E., Vachon, J. F., Paquin, P., and Fliss, Q. (2002). Effect of dynamic high pressure on microbiological, rheological and microstructural quality of Cheddar cheese. International Dairy Journal, 12, 435-446.

Knorr, D., Bottcher, A., Dornenurg, H., Eshtiaghi, M., Oxen, P., Richwin, A., and Seyderhelm, I. (1992). High pressure effects on microorganisms, enzyme activity and food functionality. High Pressure and Biotechnology, 224, 211-218.

Lau, K.Y., Barbano, D. M., & Rasmussen, R. R. (1990). Influence of pasteurization on fat and nitrogen recoveries and Cheddar cheese yield. Journal of Dairy Science, 73, 561-570.

Lee, C. H., Imoto, E. M., & Rha, C. (1978). Evaluation of cheese texture. Journal of Food Science, 43, 1600-1605.

Linnan, M. J., Mascola, L., Lou, X. D., Goulet, V., May, S., Salminen, C., Hird, D. W., Yonekura, M. L., Hayes, P., Weaver, R., Audurier, A., Plikaytis, B. D., Fanning, S. L., Kleks, A., Broome, C. V. (1988). Epidemic Listeriosis associated with Mexican-style cheese. The New England Journal of Medicine, 319, 823-828.

Linton, M., McClements, J.M.J., and Patterson, M.F. (2001). Inactivation of pathogenic Escherichia coli in skimmed milk using high hydrostatic pressure. Innovative Food Science & Emerging Technologies, 2, 99-104.

Lopez-Fandino, R., Carrascosa, A. V., and Olano, A. (1996). The effects of high pressure on whey protein denaturation and cheese-making properties of raw milk. Journal of Dairy Science, 79, 929-936.

Lopez-Fandino, R., Ramos, M., & Olano, A. (1997). Rennet coagulation of milk subjected to high pressures. Journal of Agriculture and Food Chemistry, 45, 3233-3237.

Messens, W., Estepar-Garcia, J., Dewettinck, K., and Huyghebaert, A. (1999). Proteolysis of high-pressure-treated Gouda cheese. International Dairy Journal, 9, 775-782.

Metrick, C., Hoover, D. G., and Farkas, D. E. (1989). Effects of high hydrostatic processing on heat-resistant and heat-sensitive strains of Salmonella. Journal of Food Science, 54, 1547-1549.

Moore, P.L., Richter, R. L., & Dill, C. W. (1986). Composition, yield, texture and sensory characteristics of Mexican white cheese. Journal of Dairy Science, 69, 855-862.

Molina, E., Alvarez, M. D., Ramos, M., Olano, A., Lopez-Fandino, R. (2000). Use of high-pressure-treated milk for the production of reduced-fat cheese. International Dairy Journal, 10, 467-475.

Needs, E. C., Stenning, R. A., Gill, A. L., Ferragut, V., & Rich, G. T. (2000). High-pressure treatment of milk: effects on casein micelle structure and on enzymatic coagulation. Journal of Dairy Research, 67, 31-42.

O'Reilly, C. E., O'Connor, P. M., Kelly, A. L., Beresford, T. P., & Murphy, P. M. (2000). Use of hydrostatic pressure for inactivation of microbial contaminants in cheese. Applied and Environmental Microbiology, 66, 4890-4896.

O'Reilly, C. E., Kelly, A. L., Murphy, P. M., and Beresford, T. P. (2001). High pressure treatment: applications in cheese manufacture and ripening. Trends in Food Science & Technology, 12, 51-59.

Patterson, M. F., and Kilpatrick, D. J. (1998). The combined effect of high hydrostatic pressure and mild heat on inactivation of pathogens in milk and poultry. Journal of Food Protection, 61, 432- 436.

Raso, J., Gongora-Nieto, M. M., Barbosa-Canovas, G. V., and Swanson, B. G. (1998). Influence of several environmental factors on the initiation of germination and inactivation of Bacillus cereus by high hydrostatic pressure. International Journal of Food Microbiology, 44, 125-132.

Reddy, N. R., Solomon, H. M., Fingerhut, G. A., Rhodehamel, E. J., Balasubramaniam, V. M., and Palaniappan, S. (1999). Inactivation of Clostridium botulinum type E spores by high pressure processing. Journal of Food Safety, 19, 277-288.

Richardson, G. H. (1985). Standard Methods for the Examination of Dairy Products 15th Ed. Washington DC: American Public Health Association.

Sale, A. J. H., Gould, G. W., and Hamilton, W. A. (1970). Inactivation of bacterial spores by hydrostatic pressure. Journal of General Microbiology, 60, 323-334.

Saldo, J., Sendra, E., & Guamis, B. (2000). High hydrostatic pressure for accelerating ripening of goats' milk cheese: proteolysis and texture. Journal of Food Science, 65, 636-640.

SPSS. (2000). SPSS, Inc. Chicago, IL.

Tewari, G., Jayas, D. S., and Holley, R. A. (1999). High pressure processing of foods: an overview. Science des Aliments, 19, 619-661.

Timson, W. J., and Short, A. J. (1965). Resistance of microorganisms to hydrostatic pressure. Biotechnology and Bioengineering, 7, 139-159.

Villar, R. G., Macek, M. D., Simons, S., Hayes, P. S., Goldoft, M. J., Lewis, J. H., Rowan, L. L., Hursh, D., Patnode, M., and Mead, P. S. (1999). Investigation of multidrug-resistant Salmonella serotype typhimurium DT104 infections linked to raw-milk cheese in Washington state. JAMA, 281, 1811-1816.

GENERAL CONCLUSIONS

The hand sensory evaluation method developed was proven to be useful in determining and describing cheese samples tested. Panelists were able to use the standards, the ballots, and the procedure developed. The method was efficiently utilized in grouping and describing some commercial samples, and also Queso Fresco type cheeses. Further studies and possible collaborative study are needed to confirm the method's scopes and limitations.

HHP of milk and cheese at 400 MPa for 20 minutes at ambient temperature was demonstrated to reduce microbial load. HHP treatment of cheese milk resulted in cheese with different attributes from the control. HHP treatment of cheese produced cheese with similar attributes to raw fresh milk Queso Fresco style cheese. Thus, HHP treatment of cheese might be an alternative to pasteurization that can produce safer Queso Fresco with a reduced loss of desired textural attributes.

BIBLIOGRAPHY

Allogio, V., Caponio, F., Pasqualone, A., & Gomes, T. (2000). Effect of heat treatment on the rennet clotting time of goat and cow milk. Food Chemistry, 70, 51-55.

Alpas, H., Kalchayanand, N., Bozoglu, F., Sikes, A., Dunne, C. P., and Ray, B. (1999). Variation in resistance to hydrostatic pressure among strains of food-borne pathogens. Applied and Environmental Microbiology, 65, 4248-4251.

Arias, M., Lopez-Fandino, R., and Olano, A. (2000). Influence of pH on the effects of high pressure on milk proteins. Milchwissenschaft, 55, 191-194.

Balci, A. T., & Wilbey, R. A. (1999). High pressure processing of milk- the first 100 years in the development of a new technology. International Journal of Dairy Technology, 52, 149-155.

Bell, R. A., Hillers, V. N., and Thomas, T. A. (1999). The Abuela Project: safe cheese workshops to reduce the incidence of Salmonella typhimurium from consumption of raw-milk fresh cheese. American Journal of Public Health, 89, 1421-1424.

Benito, A., Ventoura, G., Casadei, M., Robinson, T., and Mackey, B. (1999). Variation in resistance of natural isolates of Escherichia coli O157 to high hydrostatic pressure, mild stresses. Applied and Environmental Microbiology, 65, 1564-1569.

Birkkjaer, H. E., Soerensen, E. J., Joergensen, J., and Sigersted, E. (1961). The influence of the cheese making technique upon the quality of cheese. In Danish Government Research Institute for Dairy Industry, Hilleroed, Report no 128.

Bodyfelt, F.W., Tobias, J., and Trout, G.M. (1988). Sensory Evaluation of Cheese. In: The Sensory Evaluation of Dairy Products. New York: Van Norstrand Reinhold. p300-375.

Bourne, M. C. (1976). Interpretation of force curves from instrumental texture measurements. In J.M. deMan, P.W. Voisey, V. F. Rasper, and D. W. Stanley, Eds. Rheology and Texture in Food Quality (pp 244-274). Westport, CT: Avi Publishing Co.

Brennan, J.G. (1988). Texture Perception and Measurement. In: Piggott, J.R, editor. Sensory Analysis of Foods 2nd Ed. New York: Elsevier Applied Science. p69-101.

Buffa, M. N., Trujillo, A. J., Pavia, M., & Guamis, B. (2001a). Changes in textural, microstructural, and color characteristics during ripening of cheeses made from raw, pasteurized or high-pressure-treated goats' milk. International Dairy Journal, 11, 927-934.

Buffa, M. N., Guamis. B., Pavia., M., & Trujillo, A. J. (2001b). Lipolysis in cheese made from raw, pasteurized or high-pressure-treated goats' milk. International Dairy Journal, 11, 175-179.

Capella, M., Mor-Mur, M., Sendra, E., & Guamis, B. (2001). Effect of high-pressure processing on physico-chemical characteristics of fresh goats' milk cheese (Mato). International Dairy Journal, 11, 165-173.

Cheftel, J. C. (1992). Effects of high hydrostatic pressure on food constituents: an overview. High Pressure and Biotechnology, 224, 195-209.

Cheftel, J. C. (1995). Review: High-pressure, microbial inactivation and food preservation. Food Science and Technology International, 1, 75-90.

Clark, G. (1973). Staining Procedures Used by the Biological Stain Commission. Baltimore: Williams & Wilkins.

Clark, S., Warner, H., & Luedecke, L. (2001). Acceptability of Queso Fresco cheese by traditional and nontraditional consumers. Food Science Technology Int, 7, 165-170.

Corredig, M., and Dalgleish, D. G. (1996). The binding of α-lactalbumin and β-lactoglubulin to casein micelles in milk treated by different heating systems. Milchwissenschaft, 51, 123-126.

Dijksterhuis, G. B. (1997). Interpreting generalized procrustes analysis "Analysis of Variance" tables. In Multivariate Data Analysis in Sensory & Consumer Science (pp77-215). Connecticut: Food & Nutrition Press, Inc.

Drake, M.A., Harrison, S.L., Asplund, M., Barbosa-Canovas, G., and Swanson. B.G. (1997). High pressure treatment of milk and effects on microbiological and sensory quality of Cheddar cheese. Journal of Food Science 62(4): 843-845.

Drake, M.A., Gerard, P.D., and Civille, G.V. (1999). Ability of hand evaluation versus mouth evaluation to differentiate texture of cheese. Journal of Sensory Studies 14(4): 425-441.

Earnshaw, R. G. (1995). Kinetics of high pressure inactivation of microorganisms. In D. A. Ledward, D. E. Johnston, R. G. Earnshaw, and A. M. P. Hasting (Eds). High pressure processing of foods (pp 37-46). Loughborough: Notthingham University Press.

Ellenberg, L., and Hoover, D. G. (1999). Injury and survival of Aeromonas hydrophila 7965 and Yersenia enterocolitica 9610 from high hydrostatic pressure. Journal of Food Safety, 19, 263-276.

Felipe, X., Capellas, M., and Law, A. J. R. (1997). Comparison of the effects of high-pressure treatments and heat pasteurization on the whey proteins in goat's milk. Journal of Agriculture and Food Chemistry, 45, 627-631.

Fox, P. F., Guinee, T. P., Cogan, T. M., & McSweeney, P. L. H. (2000a). Bacteriology of cheese milk. In Fundamentals of Cheese Science (pp 47-50). Gaithersburg, Maryland: Aspen Publishers Inc.

Fox, P. F., Guinee, T. P., Cogan, T. M., & McSweeney, P. L. H. (2000b). Cheese rheology and texture. In Fundamentals of Cheese Science (pp 305-340). Gaithersburg, Maryland: Aspen Publishers Inc.

Garcia-Graells, C., Valckx, C., and Michiels, C. W. (2000). Inactivation of Escherichia coli and Listeria innocua in milk by combined treatment with high hydrostatic pressure and the lactoperoxidase system. Applied and Environmental Microbiology, 66, 4173-4179.

Garcia-Risco, M. R., Olano, A., Ramos, M., and Lopez-Fandino, R. (2000). Micellar changes induced by high pressure, influence in the proteolytic activity and organoleptic properties of milk. Journal of Dairy Science, 83, 2184-2189.

Gervilla, R., Ferragut, V., and Guamis, B. (2000). High pressure inactivation of microorganisms inoculated into ovine milk of different fat contents. Journal of Dairy Science, 83, 674-682.

Gervilla, R., Sendra, E., Farragut, V., and Guamis, B. (1999). Sensitivity of Staphylococcus aureus and Lactobacillus helveticus in ovine milk subjected to high pressure. Journal of Dairy Science, 82, 1099-1107.

Heinz, V., and Knorr, D. (1996). High pressure inactivation kinetics of Bacillus subtilis cells by a three-state-model considering distributed resistance mechanisms. Food Biotechnology, 10, 149-161.

Hoover, D. G., Metrick, C., Papineau, A. M., Farkas, D. F., and Knorr, D. (1989). Biological effects of high hydrostatic pressure on food microorganisms. Food Technology, 99-107.

Hwang, C.H., and Gunasekaran, S. (2001). Measuring crumbliness of some commercial Queso Fresco-type Latin American cheeses. Milchwissenschaft 56(8): 446-450.

Issanchou, S., Schlich, P., and Lesschaeve, I. (1997). Sensory Analysis: Methodological Aspects Relevant to the Study of Cheese. Le Lait: 77(1): 5-12.

Johnston, D. E., Austin, B. A., & Murphy, R. J. (1992). Effects of high hydrostatic pressure on milk. Milchwissenschaft, 47, 760-763.

Kalchayanand, N., Sikes, A., Dunne, C. P., and Ray, B. (1998). Interaction of hydrostatic pressure, time and temperature of pressurization and Pediocin AcH on inactivation of foodborne bacteria. Journal of Food Protection, 61, 425-431.

Kheadr, E. E., Vachon, J. F., Paquin, P., and Fliss, Q. (2002). Effect of dynamic high pressure on microbiological, rheological and microstructural quality of Cheddar cheese. International Dairy Journal, 12, 435-446.

Kilcast, D. (1999). Sensory Techniques to Study Food Texture. In: Roshental, A.J., editor. Food Texture: Measurement and Perception. Gaithersburg, Maryland: Aspen Publishers, Inc. p30-64.

Knorr, D., Bottcher, A., Dornenurg, H., Eshtiaghi, M., Oxen, P., Richwin, A., and Seyderhelm, I. (1992). High pressure effects on microorganisms, enzyme activity and food functionality. High Pressure and Biotechnology, 224, 211-218.

Lau, K.Y., Barbano, D. M., & Rasmussen, R. R. (1990). Influence of pasteurization on fat and nitrogen recoveries and Cheddar cheese yield. Journal of Dairy Science, 73, 561-570.

Lawless, H.T., and Heymann, H. (1999). Texture Evaluation. In: Sensory Evaluation of Food: Principles and Practices. Gaithersburg, Maryland: Aspen Publishers, Inc. p379-405.

Lee, C. H., Imoto, E. M., & Rha, C. (1978). Evaluation of cheese texture. Journal of Food Science, 43, 1600-1605.

Linnan, M. J., Mascola, L., Lou, X. D., Goulet, V., May, S., Salminen, C., Hird, D. W., Yonekura, M. L., Hayes, P., Weaver, R., Audurier, A., Plikaytis, B. D., Fanning, S. L., Kleks, A., Broome, C. V. (1988). Epidemic Listeriosis associated with Mexican-style cheese. The New England Journal of Medicine, 319, 823-828.

Linton, M., McClements, J.M.J., and Patterson, M.F. (2001). Inactivation of pathogenic Escherichia coli in skimmed milk using high hydrostatic pressure. Innovative Food Science & Emerging Technologies, 2, 99-104.

Lopez-Fandino, R., Carrascosa, A. V., and Olano, A. (1996). The effects of high pressure on whey protein denaturation and cheese-making properties of raw milk. Journal of Dairy Science, 79, 929-936.

Lopez-Fandino, R., Ramos, M., & Olano, A. (1997). Rennet coagulation of milk subjected to high pressures. Journal of Agriculture and Food Chemistry, 45, 3233-3237.

Messens, W., Estepar-Garcia, J., Dewettinck, K., and Huyghebaert, A. (1999). Proteolysis of high-pressure-treated Gouda cheese. International Dairy Journal, 9, 775-782.

Metrick, C., Hoover, D. G., and Farkas, D. E. (1989). Effects of high hydrostatic processing on heat-resistant and heat-sensitive strains of Salmonella. Journal of Food Science, 54, 1547-1549.

Molina, E., Alvarez, M. D., Ramos, M., Olano, A., Lopez-Fandino, R. (2000). Use of high-pressure-treated milk for the production of reduced-fat cheese. International Dairy Journal, 10, 467-475.

Moore, P.L., Richter, R. L., & Dill, C. W. (1986). Composition, yield, texture and sensory characteristics of Mexican white cheese. Journal of Dairy Science, 69, 855-862.

Needs, E. C., Stenning, R. A., Gill, A. L., Ferragut, V., & Rich, G. T. (2000). High-pressure treatment of milk: effects on casein micelle structure and on enzymatic coagulation. Journal of Dairy Research, 67, 31-42.

O'Reilly, C. E., O'Connor, P. M., Kelly, A. L., Beresford, T. P., & Murphy, P. M. (2000). Use of hydrostatic pressure for inactivation of microbial contaminants in cheese. Applied and Environmental Microbiology, 66, 4890-4896.

O'Reilly, C. E., Kelly, A. L., Murphy, P. M., and Beresford, T. P. (2001). High pressure treatment: applications in cheese manufacture and ripening. Trends in Food Science & Technology, 12, 51-59.

Patterson, M. F., and Kilpatrick, D. J. (1998). The combined effect of high hydrostatic pressure and mild heat on inactivation of pathogens in milk and poultry. Journal of Food Protection, 61, 432- 436.

Raso, J., Gongora-Nieto, M. M., Barbosa-Canovas, G. V., and Swanson, B. G. (1998). Influence of several environmental factors on the initiation of germination and inactivation of Bacillus cereus by high hydrostatic pressure. International Journal of Food Microbiology, 44, 125-132.

Reddy, N. R., Solomon, H. M., Fingerhut, G. A., Rhodehamel, E. J., Balasubramaniam, V. M., and Palaniappan, S. (1999). Inactivation of Clostridium botulinum type E spores by high pressure processing. Journal of Food Safety, 19, 277-288.

Richardson, G. H. (1985). Standard Methods for the Examination of Dairy Products 15th Ed. Washington DC: American Public Health Association.

Saldo, J., Sendra, E., & Guamis, B. (2000). High hydrostatic pressure for accelerating ripening of goats' milk cheese: proteolysis and texture. Journal of Food Science, 65, 636-640.

Sale, A. J. H., Gould, G. W., and Hamilton, W. A. (1970). Inactivation of bacterial spores by hydrostatic pressure. Journal of General Microbiology, 60, 323-334.

SPSS. (2000). SPSS, Inc. Chicago, IL.

Tewari, G., Jayas, D. S., and Holley, R. A. (1999). High pressure processing of foods: an overview. Science des Aliments, 19, 619-661.

Timson, W. J., and Short, A. J. (1965). Resistance of microorganisms to hydrostatic pressure. Biotechnology and Bioengineering, 7, 139-159.

Villar, R. G., Macek, M. D., Simons, S., Hayes, P. S., Goldoft, M. J., Lewis, J. H., Rowan, L. L., Hursh, D., Patnode, M., and Mead, P. S. (1999). Investigation of multidrug-resistant Salmonella serotype typhimurium DT104 infections linked to raw-milk cheese in Washington state. JAMA, 281, 1811-1816.