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Proteolysis and Lipolysis of Goat Milk Cheese[1]

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ABSTRACT

Numerous varieties of goat milk cheeses are produced worldwide. Maturation or ripening of goat and other species milk cheeses is governed by interplay of many different factors. Proteolysis and lipolysis are two major biochemical processes in the multifaceted phenomenon of cheese aging, which involves a variety of chemical, physical, and microbiological changes under controlled environmental conditions. Proteolysis of cheeses in general is influenced by several factors including plasmin, chymosin, protease from starter and nonstarter bacteria, pH and moisture levels of the curds, storage temperature and time, salt content, salt-to-moisture ratio, and humidity. Primary factors affecting lipolysis in cheeses are fatty acid composition, lipolytic enzymes, lipolytic microorganisms, moisture, temperature, storage time, oxygen, and surface area, etc. Several analytical techniques have been used to measure proteolysis of goat and (or) cow milk cheeses during ripening, such as solubility of peptides and amino acids in various solvents or precipitants, liberation of reactive functional groups, various forms of chromatography, and different forms of electrophoresis. Lipolysis of goat milk cheeses has been estimated by acid degree value (ADV), acid value, and free fatty acid concentration, while lipid oxidation of dairy goat products can be determined by peroxide value, thiobarbuturic acid value (TBA). Recent reports have shown that goat cheeses had greater rates of protein degradation than cow counterparts, and that aging time and temperature synergistically elevated most of proteolytic and lipolytic indices in goat cheeses. This paper will further discuss proteolytic and lipolytic characteristics of goat milk cheeses.

(**Key words:** proteolysis, lipolysis, goat milk cheese)

Abbreviation key: WSN = water-soluble nitrogen, **TCA-SN** = TCA-soluble N, **TBA** = thiobarbituric acid, **ADV** = acid degree value, **POV** = peroxide value.

INTRODUCTION

Goat milk cheese in the United States has gained popularity among ethnic groups, gourmet and health food lovers, and goat farmers (69), while scientific studies on characteristics of caprine cheeses including proteolysis and lipolysis are still limited.

As in cow milk cheeses, numerous biochemical and physical changes can occur in goat milk cheeses during distribution and storage processes due to ripening and degradation of nutrients in the products. Proteolysis and lipolysis are two primary processes in cheese ripening with a variety of chemical, physical, microbiological, textural, and rheological changes that take place, usually under controlled environmental conditions (9, 34, 46, 58, 59, 72, 80).

The rate, extent, and nature of protein and fat degradation of cow milk cheeses during aging have been reviewed extensively in several reports (34, 58, 95). Cheese quality is greatly influenced by levels of peptides, amino acids, and free fatty acids resulting from proteolysis and lipolysis (26, 44). Proteolysis is probably the most important biochemical event, providing a major impact on flavor and texture of most cheese varieties (34). Hydrolysis of fat catalyzed by enzymes has also been a major dairy industry problem because it can cause rancid flavor defect in milk, cheese, and other dairy products (16, 85). Flavor deterioration from lipolysis of dairy products creates serious problem in storage stability (3, 23). Although much data have been documented on the proteolysis and lipolysis of cow milk cheeses, the reports may not be directly applicable to goat milk counterparts.

The objectives of this paper are 1) to review the major factors involved in proteolysis and lipolysis in cheeses, and the methods used for analysis of the two aging parameters, and 2) to reevaluate the major highlights of recent studies on proteolytic and lipolytic characteristics of goat milk cheeses conducted mainly in our laboratories.

1. ORIGIN AND VARIETIES OF GOAT MILK CHEESES

Goat milk cheese originated in Mesopotamia. The milk was probably made into soft cheese, and then hard, ripened goat milk cheeses were developed later in the Mediterranean basin countries, such as Turkey, Greece, Syria, Israel, Iraq, and Iran (51).

France produces and exports many types of goat cheeses, including Crottin du Chavignol, Les Pyramides, Sainte Maure, Chabis, and Chabicou (51). The United States has imported 1100 metric tones of French goat cheeses in 1998 (8). Other successful goat milk cheese-producing countries include Greece, Norway, Spain, and Italy. No significant attention was given to the production of commercial goat cheese in the United States until 1980.

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Numerous varieties of goat milk cheese are produced throughout the world, depending on diversity in locality, milk composition, and manufacturing techniques used. The USDA Agricultural Handbook No. 54 (82) describes over 400 varieties of goat cheeses and lists 800 names of cheeses made from goat milk or combinations of goat with other species milk. The varietal differences among goat milk cheeses are mainly attributed to the nature of physical and chemical changes during ripening (35, 36), which are also influenced by chemicals, cultures or flavor ingredients added to curd during processing (50, 62).

The goat cheese industry is classified as a "cottage industry' due to the high moisture content in caprine cheeses, and almost all French goat cheeses are of the natural drainage type by slow coagulation (60). A recent study of commercial goat cheeses produced in the United States has shown that 20 out of 30 varieties were very high or high moisture cheeses (69).

2. PROTEOLYSIS IN CHEESE

Protein composition in the initial cow or goat milk will have profound effect on the proteolytic characteristics of ripened cheeses. Electrophoretic mobility under standard conditions shows that β-casein is the major component of casein fraction of goat milk, whereas α_{s1}-CN is the major component of cow milk (15, 45). The respective mean α_{s1}-CN and β-CN contents as a percentage of total casein in goat and cow milks are 5.6 and 38; 55 and 36 (76). Goat milk also has higher concentrations of α_{s2}- and κ-CN than cow milk. Because of these compositional differences, aging and proteolytic properties of the two species milk cheeses would be significantly different.

The firmness of cheese curd is strongly correlated with α_{s1}-CN content of milk (45, 76). β-Casein is shown to be more resistant to enzymatic degradation than α_{s1}-CN. The rate of protein degradation is strongly associated with the accessibility of proteolytic enzymes to the cheese substrates, which is governed by the structure and configuration of the cheese protein matrix. Proteolysis in cow cheese has been divided into three phases, such as before, during, and post manufacture of cheese (34), which may be applicable to goat milk cheeses.

Proteolysis Before Manufacture

Proteolysis can occur in the initial milk before cheese manufacture. This stage of proteolysis is attributable to two types of enzymes, mainly microbial and indigenous milk proteinases. Psychrotrophic bacteria are found in raw and pasteurized milk. Psychrotrophic bacteria dominate the microflora of milk cooled on-farm before collection, during transportation, and during storage at the dairy plant (34, 61). Psychrotroph populations more than 10^6 cfu/ml are likely to cause reduced recovery of milk solids as cheese, higher moisture contents, pasty texture, and off-flavors in the cheese (17, 41).

Many indigenous proteinases are present in milk. The principal proteinase is plasmin, formally known as alkaline milk proteinase, which is dissociated with casein micelle (59), and preferentially hydrolyzes β-CN to γ-CN and proteose peptones (34). Most of the proteose-peptone fraction formed by plasmin is lost in acid or rennet whey (57, 59). Plasminogen is present as the precursor of plasmin, which hydrolyzes α_{s2}-casein rapidly, but has little effect on κ-casein that is beneficial for milk stability. α_{s1}-Casein may be hydrolyzed slowly to λ-casein (1).

Proteolysis During Manufacture

Milk proteins undergo enzymatic coagulation during cheese manufacture. Rennet is a mixture of chymosin, pepsin, and other enzymes from calf stomach. Rennet coagulates milk proteins, which causes proteolysis by the formation of para-κ-CN and NPN.

Rennet coagulation is a two-stage process (34). The first involves the enzymatic formation of para-κ-CN and peptides, and the second involves the precipitation of para-κ-CN by calcium ion at temperature above 20°C. Free para-κ-CN is readily attacked by many enzymes, contributing to a well-ripened cheese (50). More than one peptide is produced for this proteolysis, and the substrate is α-CN, not β-CN (2). κ-Casein is responsible for micelle stability, but its stabilizing properties are lost at renneting (50, 97), and κ-CN is hydrolyzed during the primary phase of rennet action (96). For the secondary (nonenzymatic) phase of coagulation, casein micelles begin to aggregate when about 85% of κ-CN is hydrolyzed (22).

Proteolysis Postmanufacture

Ripening after manufacture causes many changes in the body and flavor of a cheese. Amino acids, fatty acids, methyl ketones of different carbon length, lactones, and many other organic compounds become free in the cheese and concentrate, depending on particular cheese types and aging conditions (50).

Ripening of cheese also causes two distinct phases texture development. The first phase occurs in the first 7 to 14 d, when the rubbery young cheese curd is rapidly changed to a smoother, more homogeneous texture (59). The casein network at this phase is considerably weakened through the hydrolysis of about 20% of the single bond in the α_{s1}-CN to produce α_{s1}-I by the coagulant (18). For the second phase, a more gradual change occurs in texture because of the degradation of the rest of the α_{s1}-CN during an extended aging period.

Proteolysis affects cheese ripening in several ways: 1) changes in texture through degradation of the protein network, increase in pH, and higher water binding by the newly formed amino and carboxyl groups (59), 2) development of flavor components such as amino acids and peptides, some of which may cause off-flavors (i.e., bitterness) (63), 3) changes in pH by the formation of NH_3 (49), and 4) higher release of sapid compounds during mastication (64).

Desirable and Undesirable Proteolytic Agents in Cheese Ripening

There are at least five proteolytic agents involved in cheese aging. The five proteolytic agents include: a) indigenous enzymes in raw and (or) pasteurized milks, b) coagulating enzymes, rennet, or its substitute such as chymosin, pepsin or microbial proteinases, c) starter culture bacteria and their enzymes after the cells are lysed, d) enzymes from secondary starters, such as propionic acid bacteria, and yeasts and molds, and e) nonstarter bacteria opportunistically entered during cheese making (34). Some organisms cause undesirable development of open texture and gases in the cheeses.

Heterofermentative lactobacilli, yeasts and molds and citrate- and spore-forming bacteria are undesirable organisms causing defects of ripened cheeses. They are adventitiously introduced with the starter culture; some survive pasteurization of milk or gain access to cheese milk or curds from dairy processing equipment and utensils during manufacture (56).

MAJOR FACTORS INFLUENCING THE RATE OF PROTEOLYSIS

The rate of proteolysis in cheese is highly dependent on the microbial constituency and the interplay of many other factors (50, 57, 59). Proteolytic activity in cow milk cheeses can be mainly evaluated by the type of coagulant used, existence of residual rennet and native milk proteinases, pH of cheese curd at draining and milling, salt to moisture ratio, temperature of ripening, changes in pH during ripening, redox potential, and mineral (calcium, copper, zinc, and iron) contents of the cheese (34, 50, 57, 59). The major factors affecting proteolysis in cheeses can be summarized as follows:

Enzymes (rennet and chymosin) from the starter culture. The more rennet is retained in the curd, the greater the proportion of α_{s1}-CN is hydrolyzed by the chymosin in rennet (20).

Enzymes from nonstarter and cheese milk (plasmin and other native proteinases). Cow milk contains many proteinases, the principal one being plasmin, which hydrolyzes β-CN to γ-CN and proteose peptones. Proteinases and peptidases also hydrolyze α_{s1}- and α_{s2}-CN. Most of the proteose peptone fractions are lost in acid or rennet whey (57, 59).

Moisture level of curd. The rate of ripening is proportional to the moisture content of cheese, while the duration of ripening is more or less inversely proportional to the moisture content. Small changes in the moisture to casein ratio can markedly change the availability of moisture, because a significant amount of the moisture is bound to the caseins and their degradation products (59).

pH of curd. The shift in pH reflects marked changes in the nature of newly formed compounds in cheese. The overall extent of proteolysis is increased markedly in simulated cheese at pH greater than 5.8 (66). The pH is increased at later stages of ripening due to the generation of ammonia (50). The degradation rate of α_{s1}-CN was relatively greater at low pH than that of β-CN. β-Casein was more degraded than α_{s1}-CN at pH above 5.6, presumably as a result of elevated plasmin activity (59).

Salt-to-moisture ratio. Salt content, method of salting, and salt-to-moisture ratio in curd all markedly affect the rate of proteolysis of cheese. An inverse relationship was observed between the degradation rates of both α_{s1}- and β-CN and the S:M ratio (88).

Aging time and temperature. The higher the temperature, the greater is the extent of casein hydrolysis and change in texture. Cheddar cheese ripened at 15°C develops a texture in 8 wk equivalent to that developed in 16 wk at 8°C (30). At a temperature between 2 and 10°C, the texture of Cheddar cheese will not be markedly changed, because α_{s1}-CN is considered a far more important structural element in the cheese framework than β-CN or the other caseins (25). Proteolysis of cheese is inversely correlated with cheese firmness and springiness, whereby softening of the cheese occurs as the protein matrix is degraded (30). Several recent studies in our laboratory revealed that proteolysis in goat milk cheese is synergistically elevated by the increased temperature and aging time (47, 72), which will be further discussed in the later part of this paper.

Humidity. Different types of cheese require different temperatures, relative humidities (RH) and times for ripening. For the soft goat milk cheese drying process, an average temperature of 15°C combined with 85% RH is usually satisfactory in air-conditioned rooms (60). The proper ripening of goat milk cheeses can be achieved at the temperature range of 8 to 12°C, with relatively high humidity ranging from 85 to 95% (60).

ANALYTICAL METHODS MEASURING PROTEOLYSIS IN CHEESE

Several approaches have been adopted for quantitative measurement of proteolysis of cheese during ripening. Four major methodologies include: a) solubility of peptides and amino acids in various solvents or precipitants, b) liberation of reactive functional groups, c) several forms of chromatography, and d) electrophoresis (34, 46).

Solubility of peptides in various solvents or precipitants. Nitrogen solubility under defined conditions such as fractional precipitation or solubilization is most widely used method of estimating proteolysis (62). The solubility was measured in 5% NaCl (37); sodium acetate buffers, pH 4.6 (21, 68); citrate buffers, pH 4.4 (92); 2, 2.5, 4, 10, or 12% TCA (53, 68, 71, 76); 50% ethanol (78); 70% ethanol (49); 80% ethanol plus 75% acetone (74); 5% phosphotungstic acid (75); and 0.85% picric acid (77).

Liberation of reactive functional groups. Protein degradation may also be measured by monitoring the liberation of amino or carboxyl groups in cheese through reaction with trinitrobenzene sulphonic acid (81) or ninhydrin (68). These reagents can determine a direct consequence of proteolysis by forming amino groups with cheese or its fractions, and are not dependent on an indirect effect of solubility in some particular solvents (34).

Another method assaying reactive functional groups is measuring certain amino acids using colorimetric procedures. Total tyrosine liberated in aging measured by colorimetric method was more sensitive than by the soluble nitrogen method (55). Measurement of the tyrosine content of alcohol-, TCA-, or water-soluble extracts is a well-established method of assessing proteolysis. Soluble tyrosine and tryptophan in cheese were also measured by absorbance at 270 and 290 nm (91).

Chromatographic methods. Various types of chromatographic methods have been applied to study fractionation of proteolytic degradative products in cheeses. Those methods include: 1) paper chromatography (52); 2) thin layer chromatography (27, 93); 3) ion-exchange chromatography (42); 4) HPLC (6, 10, 13, 14, 43, 92); 5) hydrophobic chromatography (54, 95); 6) gel permeation chromatography (6, 27, 33, 42); 7) cellulose derivatives(19, 55); 8) silica gel chromatography (94).

Electrophoresis. Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) has been widely used to study casein hydrolysis and the type of proteolysis in cheese, because it has high resolution and can give quantitative results (20, 24, 47, 84, 88), while its quantitative usage was criticized once (33). Other types of PAGE were also used to improve the efficiency of the gel procedures, including different buffers and staining methods (24, 60, 84).

3. LIPOLYSIS IN CHEESES AND OTHER DAIRY PRODUCTS

Lipolysis in Milk

Goat milk contains higher concentrations of short- and medium-chain fatty acids (C_6 to C_{12}) than cow milk (39, 45, 71), and the former has smaller fat globule size than the latter

(3.49 vs. 4.55) (45, 71, 76). The naturally homogenized goat milk may have different lipolytic characteristic compared with cow milk. The higher concentrations of short-chain free fatty acids in goat milk appear to be associated with the development of goaty flavor in its products. The higher levels of moisture and short-chain fatty acids in goat cheeses are likely to have greater lipolytic effects than cow counterparts.

Induced lipolysis. Several factors influence the induced lipolysis in cow milk. Those are: 1) processing factors such as agitation and foaming, homogenization, activation by temperature changes, freezing and thawing; 2) temperature during transportation, storage and processing; 3) farm factors such as milking machines, pipelines, pumping, bulk tank, storage; 4) dairy plant factors, such as mixing, separation, and poor refrigeration (26).

Spontaneous lipolysis. Spontaneous lipolysis can occur through two main factors; 1) milk processing factors, such as cooling, mixing, and separation, which disrupt milk fat globule membranes; 2) milking animal factors such as stage of lactation, feed, season, breed, mastitis, milk and fat yield, and physiological factors (estrous cycle influence the levels of milk lipase).

Microbial lipolysis. Many microorganisms that contaminate dairy products are lipolytic, produce lipase, and can cause the development of rancid flavor. The psychrotrophic bacteria are most common sources of these lipases. Bacterial lipases are different from milk lipases, are not inactivated by pasteurization, and can attack the intact fat globules in milk (26).

Factors Affecting Lipolysis and Lipid Oxidation in Cheese

The composition and integrity of lipid moieties are important for the final quality and aging properties of goat or cow milk cheeses. Goat milk contains significantly higher levels of short- and medium-chain fatty acids than cow milk (39, 71), which would have different characteristics of lipolysis and lipid oxidation in relation to the distinct flavor of goat cheeses. This situation requires detailed discussions on factors affecting lipolysis and lipid oxidation in goat milk cheeses as follows:

Free fatty acids. Positive correlations have been found between lipolyzed flavor, fat acidity, and short-chain free fatty acid contents (48). The presence of large amounts of free acids can facilitate the rate of lipid oxidation (31), and free fatty acids oxidize slightly greater rate than esterified to glycerol. Even-numbered fatty acids from butyric to lauric account for the major contribution to rancid flavor (83).

Lipolytic microorganisms and enzymes. The presence of free fatty acids in aged cheese is largely due to the action of intracellular bacterial lipases on cheese fat (40), which may not be applicable to certain varieties such as blue and Parmesan cheeses. Thermoduric bacterial lipases are responsible for developing free fatty acids in cheeses. Thermoduric bacteria survive during pasteurization, which include *Micrococcus, Microbacterium, Streptococcus, Lactobacillus*, the coryneform bacteria, *Bacillus, Clostridium,* and, often, gram-negative rods (89).

Temperature. The rate of lipid oxidation usually increases as the temperature increases (31). Temperature accelerates cheese ripening including lipolysis (16, 46)

Storage time. Lipid oxidation is also elevated with storage time. A synergistic effect of storage time and temperature on lipid oxidation and increased ADV values was observed in the recent studies with several varieties of goat cheeses (16, 46, 47).

Oxygen concentration. Bacteria protest against oxidation by competing and produce reducing substances in the milk. The control of bacterial growth not only eliminates antioxygenic effects but leads to prolonged storage time of milk (85). However, the redox system of milk would be much different from that of milk. If the oxygen supply is unlimited, the rate of lipid oxidation is independent of oxygen concentration, while the rate becomes quite proportional to oxygen concentration if oxygen concentration is very low (31).

Moisture content. The oxidation rate of lipids strongly depends on water activity (31, 53). In dry milk products, oxidation proceeds very rapidly with very low moisture content ($A_w < 0.1$). However, if the water content is increased to A_w of about 0.3, lipid oxidation become a minimum rate (31). At a higher water activity, the oxidation rate can be increased if there is any catalyst present in the product.

Antioxidant and pro-oxidant. A specific association between copper and ascorbic acid in milk increases its prooxidant properties (86). Antioxidants are substances that can delay the onset or slow the rate of oxidation of cheese lipids, which include butylated hydroxy anisole (**BHA**), butylated hydroxytoluene (**BHT**), tertiary butylhydroquionone, and propylgallate (31).

Analytical Methods Measuring Lipolysis in Cheese

Free fatty acid (FFA) values. FFA contents of goat milk cheeses can be determined by the following three methods:

a. Acid degree value (ADV); it can measure rancidity of milk products by de-emulsification and separation of free fat with detergent heat and centrifugation, and followed by titration of the free fatty acid in a weighed portion of fat with alcoholic KOH (79). The ADV value of 1.5 or greater is considered as extremely lipolyzed.

b. Acid value; this is the number of milligrams of KOH necessary to neutralize the free acids in 1 g of sample (5). The acid value may be directly converted by means of suitable factor to percent free fatty acid.

c. Total free fatty acid value; it can be determined by silica gel, gas chromatography, and HPLC methods (28, 98).

Thiobarbituric acid (TBA). The TBA value represents milligrams of malonaldehyde formed per 1000 g of sample (87), which indicates the extent of lipid oxidation. Oxidation products of unsaturated systems produce a color (red) reaction with TBA. The chromagen results from condensation of two molecules of TBA with one molecule of malonaldehyde (31).

Peroxide value (POV). The POV is determined through the titration of lipid extract with a few drops of starch indicator by sodium thiosulfate standard solution (4). The POV value is usually expressed in terms of milliequivalents of oxygen per kilogram of fat.

4. RESEARCH ON PROTEOLYSIS AND LIPOLYSIS IN GOAT MILK CHEESES

Proteolysis of Goat Milk Cheeses

Among the aforementioned analytical methods, in our recent studies, we have evaluated the extent of proteolysis by several methods, including water-soluble nitrogen (**WSN**), NPN, SDS-PAGE, and densitometry.

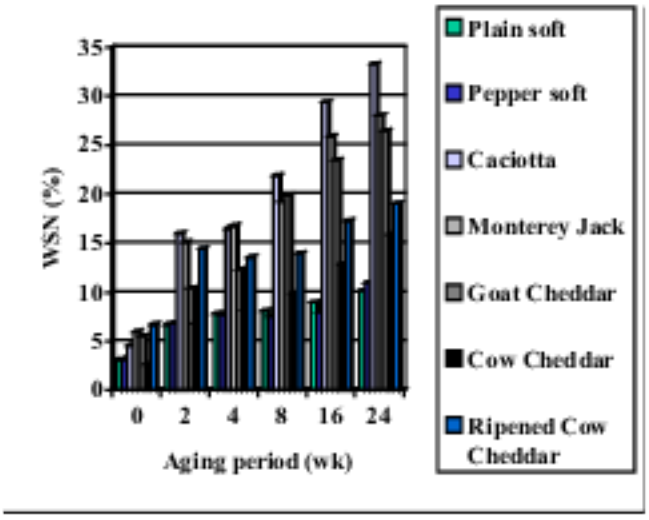

Figure 1. Profiles of water-soluble nitrogen (WSN) concentrations in the five varieties of goat milk cheese and cow milk Cheddar cheese ripened at 4°C for the 6 mo of the experimental period. The different bars represent the six cheese varieties as shown in the legend (47).

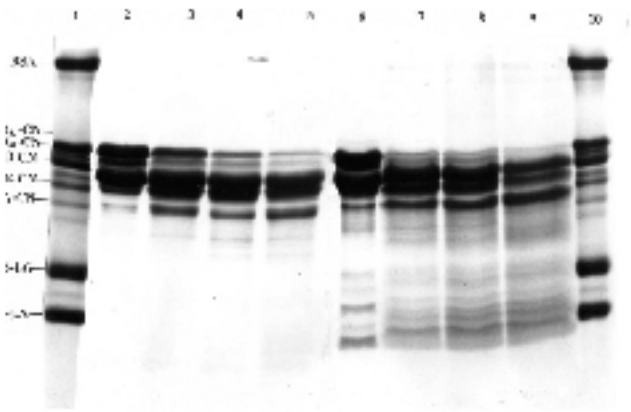

Figure 2. SDS-PAGE patterns of proteins in cow milk Cheddar cheeses aged at 4 and 13°C for 24 wk. Lanes 1 and 10 illustrate protein standards (in descending order): BSA, α_{s2}-CN, α_{s1}-CN, β-CN, κ-CN, γ_1-CN, β-LG, and α-LA. The commercial α_{s2}-CN and γ_1-CN standards were not available. Lanes 2, 3, 4 and 5 represent degradation patterns of protein samples from Cheddar ripened at 4°C for 0, 8, 16 and 24 wk, respectively. Lanes 6, 7, 8 and 9 display degradation patterns of protein samples from Cheddar ripened at 13°C for 0, 8, 16 and 24 wk, respectively (72).

Proteolysis of five varieties of goat and two cow Cheddar cheeses was evaluated in a 7 × 3 × 6 factorial experiment. The seven cheeses in two replicates were assigned to three temperature (4, 13, and 22°C) and six storage periods (0, 2, 4, 8, 16, and 24 wk). Caciotta cheese was made with rennet coagulation at 38°C, thermophilic starter, and brine salting for 12 h (47). The two cow Cheddar cheeses were fresh and 2 month old at the beginning of the experiment.

WSN. All three hard goat cheeses aged at 4°C for 6 mo had greater WSN contents ($P < 0.01$) than the fresh cow Cheddar cheese, while the same trend occurred for the ripened cow Cheddar only after 8 wk aging (Figure 1). As expected, the ripened cow Cheddar had 2 to 3 times greater WSN at the beginning of the aging experiment than did the fresh cheeses

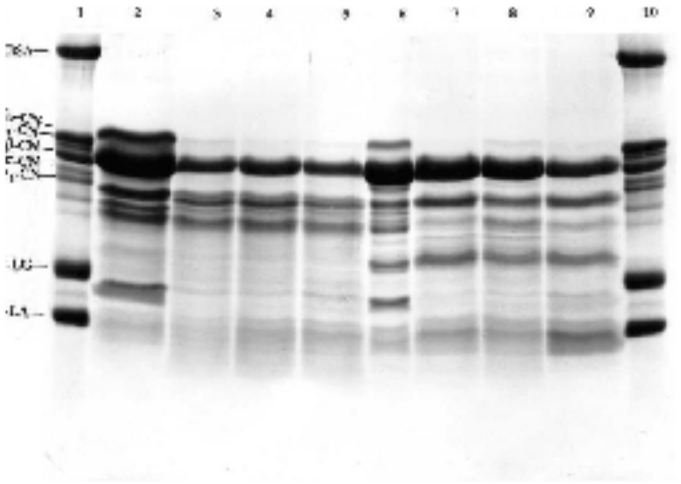

Figure 3. SDS-PAGE patterns of proteins in Caciotta and Monterey Jack goat milk cheeses aged at 13°C for 24 wk. Lanes 1 and 10 illustrate protein standards (in descending order): BSA, α_{s2}-CN, α_{s1}-CN, β-CN, κ-CN, γ_1-CN, β-LG, and α-LA. The commercial α_{s2}-CN and γ_1-CN standards were not available. Lanes 2, 3, 4 and 5 represent degradation patterns of protein samples from Caciotta cheeses ripened at 13°C for 0, 8, 16 and 24 wk, respectively. Lanes 6, 7, 8 and 9 display degradation patterns of protein samples from Monterey Jack cheeses ripened at 13°C for 0, 8, 16 and 24 wk, respectively (72).

(46). However, the two soft goat cheeses showed less WSN ($P < 0.01$) than the cow Cheddar, which may account for the acid coagulation with small amount of rennet applied to the manufacture of the soft cheeses. The soft goat cheeses also contained lower initial protein ($P < 0.01$) than the hard ones.

NPN or TCA-SN. The elevation of proteolysis was also signified by the significant increases in NPN contents in the seven cheeses aged at 4°C for the 6-mo period. The proteolytic pattern of NPN was similar to those of WSN with a few exceptions, where the causes of the drops in NPN at 4 wk were not known. In contrast, all cheeses aged at 13 or 22°C revealed increases in NPN at 4 wk and steadily elevated toward the end of the experiment (46, 47).

The correlations between pH and WSN and between pH and TCA-SN were positive and significant ($P < 0.05$ or 0.01) as all the experimental cheeses matured, while r values between the ADV values and the three variables were generally low and negative with a few exceptions (Table 1).

SDS-PAGE. All varieties of the original fresh goat cheeses showed clearly lower intensities of α_{s1}-CN bands than those of cow milk Cheddar, while intensities of β-CN in goat cheeses were much greater than those of cow Cheddar (47). The presence of α_{s2}-CN bands in goat milk Caciotta and Monterey Jack cheeses and absence of this band in cow milk Cheddar cheese aged at 13°C are also clearly demonstrated in Figures 2 and 3. The proteolytic rates of α_s-CN in both cheeses were much greater than those of β-CN. The α_{s2}-CN of goat Cheddar completely disappeared after 6 mo of aging at 4°C, whereas α_{s1}-CN in cow Cheddar was retained but became very faint at the same age (47).

Cow milk Cheddar ripened at 4 and 13°C showed the existence of a distinct protein band between α_{s1}-CN and β-CN, which was not discernible for the cow Cheddar at 22°C and almost absent in all goat Cheddar treatments (47). This band was shown to be the α_{s1}-I-CN (19), where the α_{s1}-CN was com-

Table 1. Correlation coefficients (r) between levels of measured proteolytic indices in cheeses for 6 mo of storage period.[1,2,3]

	CA-SN	pH	ADV
WSN	0.831**	0.366	0.354
	0.782**	0.840**	-0.444
	0.987**	0.899**	-0.556*
	0.984**	0.971**	-0.492
TCA-SN		0.291	0.384
		0.607*	-0.068
		0.932**	-0.522
		0.946**	-0.398
PH			0.218
			-0.690**
			-0.322
			-0.423

[1]Jin and Park, 1995 (46).
[2]Correlation coefficients for the first, second, third, and fourth rows represent the initial, 4, 13, and 22°C treated groups across all variety and storage periods.
[3] The number of observations for each r value was 84. TCA-SN: TCA-soluble N; WSN: water-soluble N; ADV: acid degree value.
*significant at 5% level; **significant at 1% level.

Table 2. Water-soluble nitrogen (WSN) and densitometric (DST) values of β-CN in cheeses aged at 4°C for the 6-mo experiment.[1,2]

	Storage period (week)			
Values	0	8	16	24
Plain soft				
WSN[3]	3.16	8.15	8.97	10.2
DST[4]	4417	6710	1861	2352
Pepper soft				
WSN	3.13	7.56	7.98	11.0
DST	3554	4598	5110	1979
Goat Cheddar				
WSN	5.53	19.9	23.5	26.5
DST	8108	6082	3338	2003
Cow Cheddar				
WSN	2.70	9.92	12.9	15.9
DST	2447	2870	1357	1140

[1]Jin and Park, 1996 (47).
[2]Values for β-CN at 4°C only; those for 13 and 22°C and proteins were not listed due to space limits.
[3]Percentage of nitrogen soluble in water to percentage of total nitrogen of a cheese sample.
[4]Value of a β-CN which is the resultant value of integrated intensity × area for each β-CN band.

Table 3. Correlation coefficients (r) between levels of water-soluble nitrogen (WSN and) densitometric values of β-CN[1,2]

Soft cheese	r	Hard cheese	r
Plain soft		Goat Cheddar	
4°C	0.367	4°C	-0.929
13°C	0.008	13°C	-0.682
22°C	0.073	22°C	-0.909
Pepper soft		Cow Cheddar	
4°C	0.340	4°C	-0.719
13°C	0.606	13°C	-0.856
22°C	0.744	22°C	-0.999**

[1]Jin and Park, 1996 (47).
[2]The r values between WSN and α_{s1}-CN in cow milk Cheddar were: -0.944, 0.653, and -0.993, respectively.
**Significant at $P < 0.01$.

pletely degraded to primary degradation products as α_{s1}-I in mature cheese. The degradative patterns of Caciotta and Monterey Jack goat cheeses aged 4°C for 24 wk had sustained α_{s2}-CN bands throughout the experiment, while no α_{s2}-CN bands were found after the 8 wk of ripening at 13 and 22°C (72).

Densitometric analysis. Breakdown products of casein fractions of the experimental cheeses were quantitatively analyzed by advanced computerized densitometry (Bio-Image Whole Band Analysis; SPARC Station 10, model no. 40; Millipore, MA). The intensities of the SDS-PAGE protein fractions expressed by integrated intensity of a protein band × area of the band were continuously decreased as the aging time advanced for all cheese varieties (46, 47).

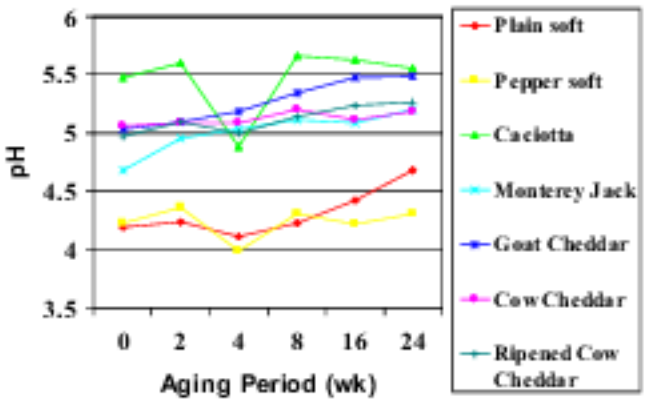

Figure 4. The pH changes of the five varieties of commercial soft and hard goat Cheeses relative to cow milk Cheddar cheese ripened at 4°C for the 6 mo of aging period (47).

Inverse correlations (r) were observed between WSN contents and densitometric values of β-CN regardless of temperature treatments throughout the experiment (Table 2). However, the β-CN values at 8 wk of aging increased except goat Cheddar which appeared to be due to the degradation of the higher molecular proteins (α_s-CN) to be the bands of lower one (β-CN). The r values for plain soft and pepper soft goat milk cheeses were positive but inconsistent, which were probably due to the lower initial protein levels of the soft cheeses compared with those hard cheeses (Table 3). The retention rate of β-CN in goat cheeses was higher than that of cow Cheddar cheese. As the levels of β-CN decreased, those of γ-CN increased, which was also observed previously (29). A concomitant decrease in β-CN bands was observed with an increase in γ-CN bands particularly γ_2-CN (29) and other low molecular-weight degradation products in all ripening cheeses (Table 4).

The elevation of γ-CN and other low molecular weight degradation products in all cheeses was highly correlated with the increase in WSN contents (47, 72). Ninety percent of the α_{s1}-CN and 70% of the β-CN present in Tybo cow milk cheeses were hydrolyzed during a 70-d aging experiment (9). The β-CN may undergo two types of hydrolysis during the first stage of ripening, one by rennet to form β-I, β-II, and β-III peptides, and one by plasmin producing γ-CN (65). The three fractions of β-CN (I, II, and III) were also identified by a later report (93).

Rheological changes by proteolysis. In a very recent rheological study with young Monterey Jack goat milk cheese in our laboratories, the young cheeses became more elastic, cohesive, meltable, viscous and softer after 4 wk of aging presumably due to proteolysis (73).

Other reports on proteolysis of goat cheeses. In a recent study on goat cheeses manufactured by two different methods: pasteurized vs. high-pressure (**HP**) treated milk (hydrostatic high-pressured non-thermal processing), cheeses from pasteurized milk had higher ($P < 0.05$) levels of both WSN and NPN fractions than those from HP-treated milk throughout the 45 d

Table 4. Comparison of densitometric values of SDS-PAGE protein bands in Caciotta cheese with those of Monterey Jack cheese from goat milk.[1]

	0 wk		8 wk		16 wk		24 wk	
Band no.	I.I × area	% I.I	I.I × area	% I.I	I.I × area	% I.I	I.I × area	% I.I
Caciotta								
1. α_{s2}	506.3	9.8	…[2]	…	40.6	3.8	4.08	0.5
2. α_{s1}	…	…	…	…	…	…	…	…
3. β	12,991	60.8	4628	60.4	2217	51.1	1511	35.8
4. κ	…	…						
5. γ1	925.6	14.7	416.9	14.9	712.3	23.5	1033.5	28.1
6.	170.4	5.8	…	…	48.4	5.0	84.5	7.6
7.	348.7	7.5	68.5	3.2	269.2	14.0	860.7	25.3
8.			674.5	15.0	6.55	0.5	13.6	1.1
9. β-LG			3.59	0.3	10.6	0.5	17.4	0.8
10.			29.5	1.2	10.2	0.7	3.68	0.2
11. α-LA			126.7	3.4	7.89	0.4	3.61	0.3
Monterey Jack								
1. α_{s2}	114.7	6.1	…	…	13.5	1.1	10.5	0.9
2. α_{s1}	…	…	…	…	…	…	…	…
3. β	6832	74.4	4277	59.9	7133	70.8	4378	73.0
4. κ	…	…	…	…	…	…	…	…
5. γ1	190.6	6.8	423.3	15.3	283.8	10.2	176.3	10.0
6.	7.61	1.0	25.9	2.2	172.2	6.0	113.9	3.9
7.	131.8	6.0	17.8	1.3	6.04	0.4	8.04	0.5
8.	114.5	4.7	542.7	15.5	177.0	7.0	144.7	7.4
9. β-LG	14.6	0.5	36.2	1.7	33.6	1.6	37.5	1.9
10. α-LA	7.48	0.3	97.2	3.8	84.8	2.9	53.1	2.4

[1]Park and Jin, 1998 (72). I.I.: Integrated intensity; Area: Area of a band. Band no.: They were named from top to bottom for those having discernible bands in the same lane.
[2]The apparent weaker bands disappeared when the negative film was developed into a photo paper.

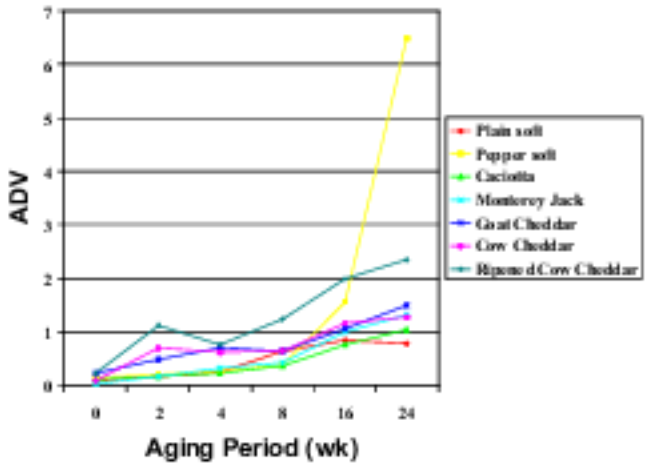

Figure 5. Comparison of mean acid degree values (ADV) among five goat milk cheeses and cow milk Cheddar cheese ripened at 4°C for the 6 mo of experimental period (47).

of ripening (90). The analysis of WSN by the cadmium-ninhydrin method (highly sensitive for free AA) showed that the HP-treated cheeses contained considerably higher ($P < 0.05$) free AA than the pasteurized cheeses for 45 d of aging.

In a 90-d aging study with Majorero goat cheese, 32.6% WSN and 20.6% NPN of total nitrogen in the cheese were evolved after 15 d of aging (32). The levels of soluble polypeptides, oligopeptides, and free amino acids were generally increased with aging times during the 90 d except a few cases, while α_s- and β-CN were decreased with the aging (32).

Lipolysis and Lipid Oxidation of Goat milk Cheeses

Two soft and three hard varieties of goat cheese and one cow Cheddar cheese were evaluated for lipolytic and lipid oxidative parameters including pH, ADV, acid value, TBA, and POV in our laboratory. All cheeses were vacuum packaged, received two temperatures (4 and 22°C) and eight aging time (0, 2, 4, 8, 12, 16, 20, and 24 wk) treatments, resulting in a 6 × 2 × 8 factorial experiment (16).

pH and TBA. The initial pH of soft cheeses were significantly ($P < 0.05$) lower than those of hard goat cheeses, while those of Caciotta cheese were highest among all the varieties (Figure 4). Although pH of soft cheeses increased with aging time, their increases were lower ($P < 0.05$) than those of hard varieties throughout the experiment. Aging time steadily elevated the pH of all varieties at 4°C, except Caciotta and pepper soft cheese at 4 wk of ripening (Figure 4). The changes in TBA (especially in soft varieties) for the whole 24-wk period resembled those in pH, which resulted in a significant positive correlation between the two parameters (16). Greater elevations in TBA were observed in soft cheeses than in hard ones, while the TBA of ripened cow Cheddar were higher than the freshly made goat cheeses throughout the study (16).

POV. The POV of soft goat cheeses increased up to 16 wk, then decreased toward the end of aging. However, POV of hard goat cheeses were not consistent up to 20 wk, and then significantly increased thereafter (16). At the end of the 6 mo of aging, the POV of cheeses stored at 22°C were generally much greater than those stored at 4°C (16).

ADV and Acid value. The ADV of the experimental cheeses steadily increased for all treatment as the aging advanced (Figure 5). The ripened cow Cheddar displayed the highest ADV throughout the experiment, while the ADV of the pepper soft goat cheese were extremely high and exceeded those of ripened cow Cheddar at 6 mo of aging (46). In the

other study (16), the acid values revealed a lack of consistency throughout the experiment, while the acid values for the 22°C group were greater than those of 4°C group.

Other reports on lipolysis in goat cheeses. For the same study with Majorero goat cheese, fatty acid composition was not significantly changed, whereas levels of individual fatty acids in the cheese were significantly increased throughout the experiment (32). Aging time greatly influenced levels of volatile FFA in goat Cheddar cheese for the first 12 wk, then remained relatively unchanged for the rest of the 24 wk aging period (7). The descending order of the relative abundance of n-chain FFA in goat Cheddar cheese were n-C_{10}, n-C_{12}, n-C_8, n-C_6, and n-C_4 (7). Although n-chain fatty acids occur in abundance, certain minor volatile branched-chain fatty acids exhibited characteristic flavors at very low concentrations (11, 12, 99, 100).

The 4-ethyloctanoic acid found to be principally responsible for the characterizing goaty-type aromatic noted in goat milk cheese (38). The presence of notable amounts of other volatile branched-chain and n-chain fatty acids provided a range of blended heavy, goaty-muttony flavors in either goat or sheep milk cheeses.

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