Effects of Frozen and Refrigerated Storage on Organic Acid Profiles of Goat Milk Plain Soft and Monterey Jack Cheeses

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ABSTRACT

The effects of 6 mo of freezing and refrigeration on organic acid profiles of 2 types of goat milk cheese [plain soft (PS) and Monterey Jack (MJ)] were studied in comparison with those of a nonfrozen control (NFC). Three lots of commercial PS cheeses were purchased, and 3 lots of MJ cheeses were manufactured at the University dairy plant. Each lot of the 2 types of cheeses was subdivided into 4 equal portions, and one subsample of each cheese was immediately stored at 4°C as the NFC for 0, 14, and 28 d. The other 3 were immediately frozen (–20°C) for 0, 3, and 6 mo (OMF, 3MF, and 6MF) and subsequently thawed the next day at 4°C. The samples were then stored at 4°C for 0, 14, and 28 d. Organic acids were quantified using an HPLC. The PS had no pyruvic acid, and MJ contained no isotartaric acid; however, several unknown large peaks appeared between propionic and butyric acids. Differences in organic acid contents between PS and MJ cheeses were significant for all acids except citric and lactic acid. Lot effect was significant for most of the known acids, indicating that variations existed in milk composition and manufacturing parameters. Effects of storage treatments (NFC, OMF, 3MF, and 6MF) were significant for most organic acids, except for orotic and a few unidentified acids. Aging at 4°C for 4 wk had little influence on all organic acids, except butyric acid. Concentrations of butyric, lactic, propionic, tartaric, and uric acids were significantly elevated as the frozen storage period advanced. At the initial stage, there were no differences in pH and acid degree values between NFC and frozen-stored groups of both cheeses. However, acid degree values gradually increased as the refrigerated storage extended up to 4 wk, indicating that lipolysis increased as the refrigeration storage at 4°C advanced. Although levels of several organic acids were changed in the goat cheeses, the prolonged frozen storage, up to 6 mo, was apparently feasible for extending storage.

Key words: goat milk cheese, freezing, refrigerated storage, organic acid

INTRODUCTION

The finer flavor and texture of cheese is developed through maturation processes that involve the interplay of biochemical (proteolysis, lipolysis, and glycolysis), physical, and microbiological changes in the cheese matrix (Law, 1984; Fox, 1989; Akalin et al., 2002; Izco et al., 2002; Park and Drake, 2005). Numerous concomitant secondary transformations also occur during cheese ripening (Law, 1984; Fox, 1989; Izco et al., 2002). The flavor profiles of cheeses are complex, variety-specific, and influenced by many substances, such as organic acids, sulphur compounds, lactones, methyl ketones, alcohols, and phenolic substances (Kosikowski, 1977; Urbach, 1993). Organic acids are important flavor compounds in most aged cheeses and are formed during hydrolytic lipolysis in cheese fat from normal bacterial growth or from the addition of acidulants during cheese manufacture (Adda et al., 1982; Park, 2001; Akalin et al., 2002; Izco et al., 2002). Quantitative determination of organic acids is an important tool for studying flavor and nutritional quality as well as being an indicator of bacterial activity of aging cheeses, as the total aroma intensity is correlated with organic acid levels in grating cheeses (Akalin et al., 2002). Many researchers have postulated organic acid content as an indicator of microbial metabolism, which can be used to classify varieties of cheese types by their aging stage (Marsili, 1985; Panari, 1986; Bevilacqua and Califano, 1989; Califano and Bevilacqua, 1999; Akalin et al., 2002). Organic acids are also major products of milk sugar catabolism by lactic acid bacteria in cheese. Nonstarter lactic acid bacteria also affect proteolysis and lipolysis during cheese aging, which in turn affect the flavor of cheese (Lane and Fox, 1996; Izco et al., 2002). Freezing cheeses is not a common industrial practice, except for a few varieties of cow milk cheeses (Kosikowski, 1977). However, Kuo and Gunasekaran (2003) suggested that interest in freezing cheese has increased as a means of prolonging desirable cheese properties. Furthermore, the seasonality of goat milk production necessitates a

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food technological approach to alternative methods for preservation of dairy goat products, whereby frozen storage of goat milk cheeses may be highly desirable for off-season marketing. In a study of freezing sheep milk, a larger ice crystal formation was observed in the ovine milk frozen at -15°C compared with -27°C (Wendorff, 2001). Some of the membranes on the milk fat globules can be damaged in the sheep milk frozen at higher temperature, which is evidenced by the higher acid degree values (ADV) in the frozen sheep milk (Anifantakis et al., 1980; Wendorff, 2001).

The objectives of this study were 1) to determine organic acid profiles of plain soft (PS) and Monterey Jack (MJ) goat cheeses and 2) to evaluate effects of 6 mo of freezing and 4 wk of refrigeration storage on organic acid contents of the 2 types of goat cheeses in comparison with those of nonfrozen control (NFC) groups.

MATERIALS AND METHODS

Preparation of Soft Goat Milk Cheese Samples

Commercial soft goat milk cheeses were purchased in 3 batches from a Grade A goat dairy in Harlem, Georgia. The soft goat cheese was manufactured using a modified method of Le Jaouen (1987). Goat milk was pasteurized at 63°C for 120 min by slow heating and slow cooling. The cheese was made by slow coagulation and natural draining. For slow coagulation, the length of time in which the curd may remain in the cheese vat varies from 12 to 48 h, depending on the type of production; the average time is about 24 h (Le Jaouen, 1987). The cheese curds then were hanged in cheese-cloth for 3 d in a cool room (22°C) before packaging. The cheeses were packaged in 454-g rod shapes with polyolefin shrink wrap and shipped to the analytical laboratory for chemical analysis in an ice-packed box via overnight delivery. Upon arrival at the laboratory, each batch of the rod-shaped soft cheeses (1 lb of 454-g packages) was cut into four 113-g pieces and vacuum-packaged before storage treatment.

Preparation of MJ Goat Cheeses

The semihard MJ goat cheese was manufactured using the bulk tank milk collected from the milking herd of midlactation Saanen, Nubian, and Alpine goat breeds at the Small Ruminant Research and Extension Center (Fort Valley State University, Fort Valley, GA). The milk was pasteurized at 63°C for 30 min, and the MJ goat cheese was processed at the University's dairy processing pilot plant according to the procedures described in Le Jaouen (1987) and Kosikowski and Mistry (1997). The MJ cheeses were initially vacuum-packaged and aged at 4°C for 6 wk to achieve optimal ripening

for consumption; then, the cheeses were subjected to the experimental storage treatments.

Experimental Design and Sample Treatments

Three lots of 2 varieties (commercial PS and manufactured MJ) of goat cheeses were assigned to 4 storage and 3 aging period treatments, which resulted in a $2 \times 4 \times 3$ factorial arrangement. Each lot of the 2 types of goat cheeses was subdivided into 4 equal portions. One subsample of the each cheese type was immediately stored at 4°C as the NFC for 0, 14, and 28 d. The other 3 subsamples were immediately frozen at -20°C for 0, 3, and 6 mo (OMF, 3MF, and 6MF) and then subsequently thawed the next day at 4°C and stored at 4°C for 0, 14, and 28 d. The OMF samples were prepared by freezing the fresh cheeses for 24 h at -20°C and then immediately thawing the next day as the frozen control treatment group. All samples taken at each treatment period were subjected to chemical and organic acid analyses.

Chemical Analysis

Basic Nutrient Analysis. Composition of basic nutrients of the goat cheese samples, such as moisture, fat, protein, and ash, were determined using procedures described in AOAC (1985) and Richardson (1985).

pH. A 10-g cheese sample and 20 mL of deionized water were placed in a Waring blender (Waring Products, Inc., New Hartford, CT) and homogenized. The pH of the homogenized cheeses was determined using an Accumet model pH meter (No. 910, Fisher Scientific, Pittsburgh, PA).

ADV. The ADV is an estimation of the amount of free fatty acids present in a fat sample, which is a quantitative index of hydrolytic lipolysis in dairy products. The ADV was determined by the procedure described in *Standard Methods for the Examination of Dairy Products* (Richardson, 1985). Fat was extracted from 10 g of grated and homogenized cheese sample in a Babcock cheese bottle. One milliliter of the extracted fat sample was titrated against the standard alcoholic 0.02 N KOH solution, and the ADV of the sample was calculated by the formula given in the same reference.

Organic Acid Analysis: Extraction of Organic Acids. All collected experimental cheese samples were frozen with liquid nitrogen and powdered using a Waring blender for homogeneity of the samples for organic acid extraction. A 3.5-g sample of frozen, powdered homogenized cheese was transferred into a 50-mL Erlenmeyer flask. Twenty milliliters of mobile phase 0.5% (wt/vol) $(\text{NH}_4)_2\text{HPO}_4$ was added to the flask and extracted for 1 h on a shaker at 400 rpm (New Brunswick Scientific, Edison, NJ); then, the extracted sample was

centrifuged at $6,000 \times g$ for 10 min. The supernatant was filtered and then filtered again through a $0.45\text{-}\mu\text{m}$ membrane filter (Supelco Inc., Bellefonte, PA). Two milliliters of the $0.45\text{-}\mu\text{m}$ membrane-filtered sample was delivered to a 5-mL syringe and filtered through a $0.45\text{-}\mu\text{m}$ membrane filter diskette (Supelco Inc.). The filtered sample was collected, and $50\text{ }\mu\text{L}$ of the finally collected sample was injected into HPLC for organic acid analysis.

Organic Acid Analysis: HPLC Analysis on Organic Acids. Final membrane-filtered liquid extracts of cheese samples were analyzed for organic acid concentrations using Hewlett Packard liquid chromatography (LC-1100 Series, Hewlett-Packard GmbH, Waldbronn, Germany) equipped with auto sampler, quaternary pump, vacuum degasser, and diode array UV detector set at 214 nm. The column used was ODS Hypersil ($5\text{ }\mu\text{m}$; $125 \times 4\text{ mm}$), column flow rate was 0.3 mL/min , and injection volume was $50\text{ }\mu\text{L}$. Solvent (mobile phase) was 0.5% (wt/vol) $(\text{NH}_4)_2\text{HPO}_4$. All organic acid standards were purchased from Sigma Chemical Co. (St. Louis, MO) and included acetic, butyric, citric, formic, lactic, malic, isomalic, orotic, propionic, pyruvic, tartaric, isotartaric, and uric acids. Individual organic acids were quantified on the basis of the external standard method. An example of quantification of various peaks of organic acids by HPLC chromatogram for PS goat milk cheese is shown in Figure 1.

Statistical Analysis. All data collected from the freezing and refrigeration storage treatments were analyzed by ANOVA; correlations between parameters, Duncan's multiple mean comparison, and least squares mean comparison of organic acids among treated goat cheeses were as described by Steel and Torrie (1960). All data were also analyzed using GLM of SAS (1990).

RESULTS AND DISCUSSION

The basic nutrient compositions of the PS and MJ cheeses are shown in Table 1. The moisture content of PS cheese was significantly ($P < 0.05$) higher than that of the MJ cheese; whereas the PS cheese was manufactured by natural draining, the MJ cheese was made by a pneumatic cheese press overnight. The MJ cheese had higher protein, fat, ash, and total solids contents than the PS cheese.

The profiles of the mean organic acid contents of PS and MJ goat cheeses after 6 mo of frozen storage compared with the NFC groups are shown in Table 2. One of the most remarkable observations on organic acid profiles of the 2 cheeses was that tartaric and pyruvic acids were absent in the PS cheese, and isotartaric acid was absent in the MJ cheese. In addition, although most known organic acids in both cheeses throughout

the storage experiment were consistently detected, a few other known organic acids were nondetectable for certain storage periods, such as orotic acid in PS cheese and tartaric, malic, and pyruvic acids in MJ cheeses (Table 2). All of the nondetectable levels of organic acids generally were observed after freezing or frozen storage treatments, except tartaric acid in MJ cheese. There were a few unknown organic acid peaks detected consistently in significant amounts in both PS and MJ goat cheeses (Figure 1).

As shown in the profiles of organic acid concentrations in Table 2 and in an example chromatogram of the organic acids in Figure 1, lactic acid concentrations were highest among all known organic acids for both PS and MJ cheese at the initial stage prior to storage and aging treatments, except butyric acid in MJ cheese. Akalin et al. (2002) reported that lactic acid accounted for approximately 95% of the total organic acid content in their ripening pickled white cheese study, where a similar trend was observed in our goat cheese study. The respective lactic acid concentrations in their study represented 76, 69, and 60% of total acids after 2, 9, and 12 mo of aging, whereas lactic acid levels of the initial PS and MJ goat cheeses in our study were 51 and 37%, respectively.

Lactic acid contents of PS cheese in this study showed a drop at initial freezing and then increased thereafter during frozen storage (Table 2; Figure 2). Lactic acid levels in MJ cheese were not consistent as in PS cheese; they decreased up to 3 mo in frozen storage and then considerably ($P < 0.05$) increased at 6 mo of frozen storage (Table 3; Figure 2). Lactic acid contents in both PS and MJ cheeses at 6 mo of frozen storage were significantly ($P < 0.05$) greater than those of 3 other storage groups (Tables 2 and 3). The main function of a starter culture is to convert lactose to lactic acid at an accelerated rate in the early stages, as lactic acid can inhibit unwanted contaminating organisms such as coliforms (Fox, 1989). The formation of lactic acid is essential for optimal manufacture of cheese, flavor development, proper aging, and achieving a desirable quality of the final product (Wong, 1974).

Butyric acid contents of PS cheese for all storage treatments were low compared with those of lactic acid and similar to the levels of other organic acids (Tables 2 and 3; Figure 3). However, there were substantial ($P < 0.001$) elevations in butyric acid content of MJ cheese for all nonfrozen and frozen storage treatment periods compared with those of PS cheeses (Tables 2 and 3; Figure 3). The compositional differences, such as in total fat and total solids levels between MJ and PS cheeses (Table 1), might have accounted for the high elevation of butyric acid in MJ cheese. These extremely high levels of butyric acid in MJ cheese accounted for

Figure 1. Organic acid peaks of plain soft goat milk cheese as illustrated by HPLC chromatogram. The peak numbers correspond to 1) tartaric, 2) formic, 3) malic, 4) lactic, 5) acetic, 6) orotic, 7) citric, 8) uric, 9) isomalic, 10) propionic, and 11) butyric acids.

the 6-wk preripening treatment before the actual frozen storage experiment in this study. The extent of elevation for the 6MF group was much greater than the other frozen storage and NFC groups, where the ranges of increase were 6 to 16 times higher than the levels in corresponding groups of PS cheese (Tables 2 and 3; Figure 3).

Butyric and propionic acid levels in PS cheese were increased as the storage time progressed; in MJ cheese, the increases were substantial in butyric acid, but were lower and less consistent in propionic acid (Table 2; Figure 3). Our results are in agreement with Akalin et al. (2002); those researchers observed butyric acid levels at 0, 18, 20, and 27% of the total organic acid content in their pickled white cheese for the periods of 0, 6, 9,

and 12 mo of aging, respectively. A rapid increase in butyric acid content appeared to be responsible for higher organic acid concentration, especially for the later several months. Other researchers (Bevilacqua and Califano, 1992; Lombardi et al., 1994; Fedio et al., 1994) also observed similar phenomena of lactic, propionic, and butyric acid concentrations in Port Salut Argentinio, Reggianito, and Swiss cheeses. Butyric and propionic acid fermentations are the result of the lipolytic and proteolytic activities of starter bacteria and numerous secondary microflora (Law, 1984; Fox, 1989; Lane and Fox, 1996).

Differences in propionic acid between cheese type, storage periods, and type $\times$ storage periods were significant ($P < 0.01$ or 0.05). Propionic acid content in PS

Table 1. Basic nutrient composition (% wet basis) of plain soft (PS) and Monterey Jack (MJ) goat milk cheeses¹

Cheese	Moisture	Protein	Fat	Carbohydrate	Ash	Total solids
PS	60.1 ^a	14.5 ^b	19.4 ^b	4.34	1.66 ^b	39.9 ^b
MJ	42.1 ^b	27.3 ^a	23.4 ^a	3.05	4.05 ^a	57.8 ^a

^{a,b}Means with different superscript are significantly different ($P < 0.05$).

¹Each value is a mean of 3 batches of the 2 cheeses. Each batch was analyzed at least in triplicate.

Table 2. Comparison of organic acid contents among nonfrozen (NFC) and 0-, 3-, and 6-mo frozen-stored (OMF, 3MF, 6MF, respectively) plain soft (PS) and Monterey Jack (MJ) goat milk cheeses aged at 4°C for 4 wk^{1,2}

Organic acid	NFC			OMF			3MF			6MF		
	0 d	14 d	28 d	0 d	14 d	28 d	0 d	14 d	28 d	0 d	14 d	28 d
Tartaric acid (TA)												
PS (Iso-TA)	0.86	0.93	0.93	0.75	0.67	0.70	1.53	1.49	1.42	1.62	1.79	1.92
MJ (TA)	— ⁵	—	0.38	—	0.34	0.49	—	0.16	0.57	0.21	0.19	0.48
Formic acid												
PS	2.32	2.23	2.21	2.66	2.60	2.59	1.63	1.74	1.81	2.31	2.37	2.61
MJ	2.59	2.62	2.62	2.63	2.40	2.44	2.72	2.41	2.10	3.00	2.73	2.18
Orotic acid ³												
PS	0.042	0.011	0.012	0.043	0.014	0.013	0.011	0.037	0.036	—	—	—
MJ	0.012	0.010	0.009	0.004	0.008	0.013	0.010	0.014	0.028	0.012	0.018	—
Malic acid ⁴												
PS	1.13	1.32	1.44	1.22	1.40	1.21	0.42	0.44	0.30	0.031	0.033	0.022
MJ	1.26	1.00	1.24	1.91	1.96	2.34	—	—	—	0.70	0.65	0.28
Lactic acid												
PS	10.04	10.22	10.37	8.96	10.15	9.83	11.19	10.97	10.70	12.47	12.30	12.91
MJ	10.89	9.56	9.16	9.72	10.00	10.24	7.02	10.29	9.12	13.05	12.14	11.05
Acetic acid												
PS	2.86	4.20	4.34	5.01	4.03	3.69	3.24	3.32	2.65	3.04	3.41	4.41
MJ	1.82	1.86	2.43	2.00	2.19	2.82	2.47	2.94	2.61	3.51	4.93	4.84
Citric acid												
PS	0.69	0.82	0.87	0.88	0.89	0.81	2.12	1.73	1.58	0.58	0.92	1.42
MJ	1.30	0.85	0.77	1.05	1.31	1.32	1.20	1.22	0.91	1.63	1.27	0.93
Uric acid ³												
PS	0.017	0.02	0.015	0.029	0.038	0.034	0.037	0.043	0.040	0.083	0.085	0.084
MJ	0.062	0.072	0.064	0.064	0.065	0.063	0.008	0.020	0.013	0.013	0.016	—
Propionic acid												
PS	0.71	0.79	0.69	1.28	0.60	0.83	1.53	2.13	2.15	5.36	4.25	4.84
MJ	0.66	3.36	5.96	6.31	5.53	7.16	2.34	3.34	3.62	4.88	4.71	3.65
Pyruvic acid												
PS	—	—	—	—	—	—	—	—	—	—	—	—
MJ	1.85	1.29	1.11	2.04	1.80	1.96	—	—	—	—	—	1.57
Butyric acid ^{3,4}												
PS	1.07	1.20	1.29	1.01	1.21	1.62	1.16	1.53	1.83	2.76	2.93	4.93
MJ	9.56	10.58	11.94	10.52	13.04	14.04	28.01	37.57	38.56	35.36	48.91	32.28

¹Some missing data indicate nondetectable levels of specific organic acids.

²Several unknown organic acids were consistently detected, but not listed in the table.

³Differences between cheese types are significant ($P < 0.05$).

⁴Differences among storage treatments are significant ($P < 0.05$).

⁵Nondetectable levels of organic acids.

cheese had continuous elevation as the frozen storage period progressed; those in MJ cheese were significantly increased, especially after freezing (OMF) (Tables 2, 3, and 5). Hough et al. (1996), studying Reggiano grating cheese, showed that the flavor descriptors (total intensity, cheesy, salty, tongue-tingling, hot, and residual intensity) were predicted from organic acids. They also noticed that propionic acid was a good indicator of flavor development, and total aroma intensity was well correlated with organic acid contents.

Acetic acid contents of the initial PS cheese variety showed a trend opposite that of the MJ variety, where NFC, OMF, and 3MF groups of PS cheese were much higher than those of MJ cheese; the opposite trend appeared for the 6MF group (Tables 2 and 3; Figure 4). The 6MF treatment of MJ cheese had greater ($P < 0.05$) acetic levels for 0, 14, and 28 d of refrigeration

aging than the other frozen storage and NFC groups (Table 3). The increase in acetic acid as well as other short-chain free fatty acids, such as propionic and butyric acids, in MJ cheese for the 6MF group would be an indication of intensity of bacterial fermentation, which occurs during ripening (Akalin et al., 2002).

Significant ($P < 0.05$) differences were found in levels of some known organic acids, such as isotartaric, formic, uric, and citric acids, between different storage treatment groups in PS cheese, but not in MJ cheese (Table 4). Several unknown organic acids in both PS and MJ cheeses also showed differences ($P < 0.05$) in their concentrations between NFC and frozen-stored groups.

Formic acid levels in PS cheese inconsistently fluctuated between storage groups (Table 4). The formic acid contents of the commercial PS goat cheeses might

Figure 2. Comparison of lactic acid contents in plain soft (PS) and Monterey Jack (MJ) goat cheeses among different storage treatments at 0 d of aging. NFC = nonfrozen control, 6MF = frozen 6 mo, 3MF = frozen 3 mo, and 0MF = frozen 0 mo.

Figure 4. Comparison of acetic acid contents in plain soft (PS) and Monterey Jack (MJ) goat cheeses among different storage treatments at 0 d of aging. NFC = nonfrozen control, 6MF = frozen 6 mo, 3MF = frozen 3 mo, and 0MF = frozen 0 mo.

have been governed by the presence of *Streptococcus salivarius* ssp. *thermophilus*, which produces formic acid from lactose (Thomas, 1985; Akalin et al., 2002). Although the commercial mesophilic culture may not contain *Streptococcus thermophilus*, possibilities can exist where *S. thermophilus* bacteria could get into the cheese manufacturing processes opportunistically through utensils and other possible contaminations in the commercial dairy goat processing plants.

Uric acid content of PS cheese was highest in the 6-mo frozen-stored group compared with the 0MF and

Figure 3. Butyric acid contents of plain soft (PS) and Monterey Jack (MJ) goat cheeses among different storage treatments at 0 d of aging. NFC = nonfrozen control, 6MF = frozen 6 mo, 3MF = frozen 3 mo, and 0MF = frozen 0 mo.

NFC groups. Similar results were observed in a cow cheese study (Akalin et al., 2002). Malic acid contents in both PS and MJ cheeses were generally decreased with extended storage periods; there was an inconsistent trend with the 0MF treatment (Table 2). Changes in orotic acid in both cheeses were also not consistent, although the effect of storage on orotic acid of PS cheese was significant, and that of MJ was nonsignificant.

Citric acid in PS cheese also fluctuated, but differences between storage groups were significant ($P < 0.05$), showing that the 3MF cheese had the highest level compared with the other storage groups (Table 4). This inconsistent change in citric acid was previously observed in Reggiano cheese (Lombardi et al., 1994) and pickled white cow cheese (Akalin et al., 2002). Because the starter bacteria *Lactococcus lactis* ssp. *cremoris* can utilize citric acid for fermentation, increases and decreases in citrate can be postulated by its biochemical metabolism, i.e., it could act as both substrate and product (Akalin et al., 2002). Citric acid can be utilized as substrate by the starter bacteria to produce pyruvic and acetic acids, which are the intermediary products of the citric acid cycle or Krebs cycle (Adda et al., 1982).

There were differences ($P < 0.001$) in pH between the 2 varieties of goat cheeses; the pH of PS cheese was lower ($P < 0.001$) than the pH of MJ for all NFC and frozen-stored groups. However, there were no differences in pH between different refrigerated aging treatments for 4 wk for both PS and MJ cheeses, regardless of storage treatment groups (Figure 5). In fact, there were no changes in pH among storage peri-

Table 3. Comparison of differences in selected mean organic acid concentrations (mg/g of cheese) between plain soft (PS) and Monterey Jack (MJ) goat cheeses during 6 mo of frozen storage relative to those of nonfrozen control (NFC) groups

Treatment ¹	Lactic		Acetic		Propionic		Butyric	
	PS	MJ	PS	MJ	PS	MJ	PS	MJ
6MF	12.56 ^a	12.08 ^a	3.620 ^{ab}	4.426 ^a	4.82 ^a	4.41 ^{ab}	3.540 ^a	38.9 ^a
3MF	10.95 ^b	8.67 ^b	3.070 ^b	2.667 ^b	1.94 ^b	3.06 ^b	1.510 ^b	34.0 ^a
NFC	10.26 ^{bc}	9.81 ^b	3.884 ^{ab}	2.048 ^b	0.69 ^c	3.48 ^{ab}	1.185 ^b	10.8 ^b
0MF	9.46 ^c	10.00 ^b	4.173 ^a	2.357 ^b	0.90 ^c	6.34 ^a	1.282 ^b	12.6 ^b

^{a-c}Means with different letters differ significantly ($P < 0.05$ or 0.01).

¹Treatment = storage treatments; 6MF = frozen 6 mo, 3MF = frozen 3 mo, and 0MF = frozen 0 mo.

ods, nor among different refrigerated aging treatments. These results show that freezing and/or frozen storage up to 6 mo does not have any impact on pH of the 2 goat cheeses, suggesting that freezing is feasible without altering pH in the goat cheeses.

The respective mean ranges of ADV for PS and MJ cheeses for all storage treatments were 0.42 to 0.93 and 1.13 to 1.46, indicating that the levels of ADV in MJ cheese were higher ($P < 0.01$) than those in PS cheese (Figure 6). The ADV of MJ cheese were expected to be much higher than those of PS cheese, as the MJ was preripened for 6 wk prior to the study. The effect of refrigerated aging for 4 wk on ADV within each storage treatment in both PS and MJ cheeses was generally increased but statistically not significant.

Although increased lipolysis (higher ADV) occurred in the MJ cheese during 4 wk of refrigerated storage, the organoleptic qualities of the cheese were acceptable in a preliminary sensory study. Freezing had minimal effect on the freshness and sensory quality of both PS and MJ cheeses, and the loss of freshness of the soft cheese was apparent after 4 wk of refrigerated storage at 4°C. The increase in ADV in both goat cheeses in this study suggests that the levels of free fatty acids, especially the short-chain acids might have been elevated, which would be responsible for the characteristic flavor of goat cheeses (Green and Manning, 1982).

Analysis of variance for main factors such as cheese type (variety), storage, and aging effects and their interaction effects on organic acid levels of PS and MJ

Figure 5. pH profile of plain soft (PS) and Monterey Jack (MJ) goat cheeses during nonfrozen (NFC) and 0-, 3-, and 6-mo frozen-storage treatments (0MF, 3MF, and 6MF, respectively) and 4 wk of aging at 4°C.

Figure 6. Acid degree value (ADV) profiles of plain soft (PS) and Monterey Jack (MJ) goat cheeses during nonfrozen (NFC) and 0-, 3-, and 6-mo frozen-storage treatments (0MF, 3MF, and 6MF, respectively) and 4 wk of aging at 4°C.

cheeses (Table 5) revealed that variety difference was most significant (mostly $P < 0.001$) except for lactic acid. Effect of storage treatments (NFC vs. frozen-stored) was also highly significant ($P < 0.001$) for acetic, butyric, citric, lactic, uric, and propionic acid, but was not significant for formic and orotic acid. Among the 3 main effects, aging effect (the refrigerated aging at 4°C for 28 d) did not show any significance on all organic acid contents.

Among interaction effects (2-way or 3-way interactions among main factors), cheese type $\times$ storage period interactions were highly significant for acetic, butyric, citric, propionic, and uric acid, but not for formic, lactic, and orotic acid (Table 5). The other 2-way interactions for type $\times$ aging and storage $\times$ aging effects were

not significant, except for orotic acid. The 3-way interaction of type $\times$ storage $\times$ aging effect was also not significant.

In our goat cheese study, freezing and frozen storage caused significant changes in some organic acids, but refrigeration aging at 4°C for 28 d showed nonsignificant effects (Table 5). Conversely, Califano and Bevilacqua (1999) found that the effect of freezing was not significant, but the effect of ripening time on organic acid contents of their cow milk Mozzarella cheese was significant. Although organic acids in cheese are produced by hydrolysis of fatty acids, bacterial growth, normal bovine metabolic processes, or direct addition of acidulants (Adda et al., 1982; Akalin et al., 2002; Izco et al., 2002), its composition in cheeses can also

Table 4. Comparison of mean organic acid concentrations (mg/g of cheese) of plain soft (PS) goat cheeses between different storage periods for pooled data across aging periods¹

Treatment ²	Isotartaric	Formic	Uric	Citric	Unknown-8	Unknown-11
6MF	1.777 ^a	2.429 ^{ab}	0.084 ^b	0.974 ^b	1.502 ^a	0.084 ^b
3MF	1.480 ^b	1.728 ^c	0.040 ^c	1.809 ^a	0.713 ^c	0.040 ^c
NFC	0.925 ^c	2.225 ^b	0.007 ^a	0.797 ^b	1.114 ^b	0.007 ^a
0MF	0.708 ^d	2.620 ^a	0.032 ^c	0.860 ^b	1.182 ^{ab}	0.032 ^c

^{a-d}Means with different letters differ significantly ($P < 0.05$ or 0.01).

¹Monterey Jack cheese did not show significant differences in the known organic acids listed.

²Treatment = storage treatments; 6MF = frozen 6 mo, 3MF = frozen 3 mo, and 0MF = frozen 0 mo. NFC = nonfrozen control.

Table 5. Analysis of variance (*F*-values) for main effects (cheese type, storage, and aging) and their interaction effects on major known organic acid contents of 2 types of goat cheeses (plain soft and Monterey Jack)¹

Effect	Acetic	Butyric	Citric	Formic	Lactic	Orotic	Propionic	Uric
Type	15.7***	163.7***	0.03	4.21*	3.33	13.6***	12.1***	19.0***
Storage	5.36***	20.2***	4.86***	2.59	8.50***	1.48	2.62*	33.0***
Aging	1.46	1.18	0.24	0.27	0.31	0.32	0.67	0.86
Type × storage	7.67***	16.2***	6.02***	2.10	2.46	0.21	3.39*	32.1***
Type × aging	0.29	1.50	1.26	0.85	0.29	3.08*	0.53	0.02
Storage × aging	1.53	0.44	0.51	0.07	0.85	3.84*	0.40	0.05
Type × storage × aging	1.14	0.71	1.48	0.37	1.41	2.91	0.38	0.10

¹Pyruvic and malic acid data are not included because of the incomplete and insignificant data.

P* < 0.05; **P* < 0.001.

be changed by different species milk as well as freezing or frozen storage.

In a frozen storage study of ovine milk cheeses to control inventory, Tejada et al. (2002) reported that lactic acid concentration and pH in fully ripened cheeses were significantly different between control cheeses and those kept in frozen storage for 9 mo. They concluded that freezing fully ripened sheep milk cheeses at −20°C for up to 6 mo was a suitable method of storing cheeses to control inventory throughout the year, which is in agreement with the results of the present goat cheese study.

Cow Cheddar cheese could be also satisfactorily frozen if cut into ≤1-lb pieces and wrapped in foil (Morris and Combs, 1955). The MJ cheese in our study was frozen using methods similar to those of Tejada et al. (2002) and Morris and Combs (1955) and were vacuum-packaged. The impact of freezing on goat cheeses at initial stages of the 3 frozen-storage treatments of this study appeared to be minimal on the sensory qualities that were shown in our recent companion study (Park and Drake, 2005).

In a study of evaluating ripening low-moisture cow Mozzarella cheese for various periods before freezing, freezing rates, and frozen storage, Bertola et al. (1996) also concluded that the Mozzarella cheese could be frozen and stored at −20°C without quality loss as long as the final product had been aged from 14 to 21 d before being consumed. The freezing rates of their experiment did not affect the functional and food quality attributes of the bovine cheese.

CONCLUSIONS

Up to 6 mo of frozen storage (−20°C) of PS and MJ goat cheeses caused some change in the level of several organic acids. However, the frozen storage treatments had minimal impact on the pH and lipolytic index of both PS and MJ cheeses. The 4-wk refrigeration storage at 4°C caused an apparent loss of freshness of the soft goat cheeses. Some changes in organic acid

contents in this study and no significant losses of sensory qualities in the frozen-stored cheeses observed in the other companion study suggest that the frozen storage technique can extend shelf-life of goat milk cheeses.

The outcomes of this study are consistent with previous studies with cow and sheep milk cheeses where frozen storage of the reported varieties of bovine and ovine milk cheeses were shown to be feasible with acceptable sensory and chemical qualities. The results of this study indicate that frozen storage of caprine milk cheeses would be feasible for later marketing, which can overcome the seasonality of goat milk production and enhance the sustainability of the dairy goat industry. However, further studies may be desired to determine the effect of frozen storage beyond 6 mo on food qualities of caprine cheeses, as well as to explore the methodologies of controlling sensory properties of goat cheeses under extended refrigeration (4°C) storage.

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