Influence of Ovine Milk in Mixture with Bovine Milk on the Quality of Reduced Fat Muenster-Type Cheese

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ABSTRACT

Reduced fat Muenster-type cheeses were manufactured from a mixture of bovine skim milk and ovine whole milk and from bovine milk only (control). Cheeses were evaluated at 15, 30, 60, 90, 120, and 180 d of age for numbers and type of microflora, casein hydrolysis, and amounts of free fatty acids. αs1-Casein degradation was similar for both cheeses during the aging period, but β-casein degradation proceeded at a faster rate in the control cheese. The total amounts of free fatty acids remained constant throughout the ripening time; however, the cheeses produced with bovine/ovine milk yielded a significantly larger amount of caprylic (C8:0) and capric (C10:0) acids compared with the bovine milk cheeses. Lactobacilli increased during the aging period, while the populations of lactic acid bacteria, yeast and molds, and lipolytic organisms did not increase. Both cheeses had comparable cheese flavor intensity, but the bovine/ovine milk cheeses had a greater occurrence of off flavors. The bovine/ovine milk cheeses were firmer than the bovine cheeses throughout the aging period.

(Key words: ovine, reduced fat, cheese)

Abbreviation key: FID = flame-ionization detector, TPA = texture profile analysis.

INTRODUCTION

In the last two decades, awareness of the link between a high fat diet and heart disease has encouraged the development of reduced fat dairy products. The development of reduced fat cheeses has been challenging. Cheese owes the major portion of its desirable flavor to the presence of fat. The flavor of reduced fat cheeses tends to be flat, bitter, meaty, and brothy (Olson and Johnson, 1990). Besides flavor defects, reduced fat Cheddar cheese has a shorter shelf life of 3 to 4 mo compared with full fat cheese that retains desirable attributes for at least 6 mo. Several approaches to improving the flavor have been attempted in the past years, either by modifying the cheese-making procedures (Chen et al., 1991) or by genetically altering the starter culture (Steele, 1998), but none of them has been truly successful.

In Europe and the Middle East, cheeses made from ovine milk are known for their high flavor intensities. Due to the high price of ovine milk in some areas, ovine milk is mixed with bovine milk to produce cheeses similar to ovine milk but at a lower cost. Ovine milk contains twice the solids of bovine milk (Anifantakis, 1986), and almost twice as much fat as bovine milk. The high fat content is responsible for the unique flavor and aroma of ovine milk cheese (Anifantakis, 1986; Kalantzopoulus, 1993). Low molecular weight FFA (C4:0 to C10:0) are more abundant in ovine milk than in bovine milk (20 to 25% vs. 10 to 12%; Anifantakis, 1986). Free fatty acids liberated during cheese manufacture and aging play an important role in the flavor of cheese and also serve as precursors for the formation of ketones (Woo et al., 1984). Varieties of cheeses that have strong flavor such as Blue or Gruyere cheeses tend to have large amounts of FFA, while those that exhibit milder flavors contain moderate amounts of FFA (Woo et al., 1984). The objective of this study was to determine whether the flavor of reduced fat cheeses could be improved with the addition of ovine milk to bovine milk for use in cheese manufacture.

MATERIALS AND METHODS

Cheese Manufacture

Reduced fat Muenster-type cheese was made from a mixture of approximately 80% bovine skim milk and 20% ovine whole milk. Midlactation ovine milk was obtained from the University of Wisconsin Experimental Station at Spooner. After milking, the raw milk was flash frozen at −27°C in 11.25-kg pails and shipped to the University of Wisconsin-Madison. The ovine milk
was stored at −27°C, until it was needed. A 25% reduced fat Muenster cheese containing only bovine milk was made as a control. Milks for cheese manufacture were standardized to a casein-to-fat ratio of 1.73, so that finished cheeses would have similar composition (Table 1). Milk was pasteurized at 71°C/16 s, cooled to 32°C, and stored at 7°C, until it was needed. A 25% reduced fat Muenster cheese containing only bovine milk was made as a control. Milks for cheese manufacture were standardized to a casein-to-fat ratio of 1.73, so that finished cheeses would have similar composition (Table 1). Milk was pasteurized at 71°C/16 s, cooled to 32°C, and stored at 7°C, until it was needed. A 25% reduced fat Muenster cheese containing only bovine milk was made as a control. Milks for cheese manufacture were standardized to a casein-to-fat ratio of 1.73, so that finished cheeses would have similar composition (Table 1).

Table 1. Mean and standard deviations of the chemical composition of bovine milk and the mixture of bovine/ovine milk used for production of reduced fat Muenster-type cheese.1

<table>
<thead>
<tr>
<th>Component</th>
<th>Bovine milk</th>
<th>Bovine/ovine milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solids, %</td>
<td>9.78 ± 0.07</td>
<td>11.48 ± 0.06</td>
</tr>
<tr>
<td>Fat, %</td>
<td>1.39 ± 0.04</td>
<td>1.73 ± 0.07</td>
</tr>
<tr>
<td>Total protein, %</td>
<td>3.08 ± 0.05</td>
<td>3.92 ± 0.12</td>
</tr>
<tr>
<td>Casein, %</td>
<td>2.38 ± 0.09</td>
<td>3.02 ± 0.08</td>
</tr>
<tr>
<td>C/F ratio2</td>
<td>1.71</td>
<td>1.74</td>
</tr>
</tbody>
</table>

1Values are means of duplicate determinations for two production days.
2All components are presented as a percentage (wt/wt).
3Ratio of casein to fat in the milk.

Compositional Analyses

Milk was analyzed for fat, protein, and total solids content (Bradley et al., 1993) as well as coliforms (Christen et al., 1993), lactobacilli, psychrotrophs, lipolytic organisms (Frank et al., 1993), and standard plate counts (Houghtby et al., 1993). The cheese quarter for compositional analyses was ground in a food mill (Black & Decker, Power Pro FP 1000, Shelton, CT). Compositional analyses of cheeses were done at 15 d of age. The percentage of moisture was determined by drying 3 g of ground cheese in predried, disposable aluminum pans covered with glass/fiber disks in a vacuum oven at 100°C for 5 h (Bradley et al., 1993). Fat content was determined by the Mojonnier method (Bradley, 1995) and protein by the Kjeldahl method (Bradley, 1995). The pH was determined using the quinhydrone/gold electrode as described by Van Slyke and Price (1979). The salt content was determined using a Chloride Analyzer (Corning, model 926; Corning, Inc., Corning, NY). All compositional determinations were carried out in triplicate.

Microbial Analysis

Coliforms (Christen et al., 1993), lactic acid bacteria, lactobacilli, lipolytic organisms, yeast, and molds (Frank et al., 1993) were enumerated in cheeses at 15, 30, 60, 90, 120, and 180 d of age. Coliforms were enumerated on violet red bile agar (Difco, Detroit, MI) at 32°C for 24 h. Total lactic acid bacteria were enumerated by utilizing Elliker agar (Difco) incubated at 37°C for 48 h under anaerobic conditions. Lactobacilli were enumerated on SL Rogosa agar incubated anaerobically at 32°C for 48 h. Lipolytic organisms were enumerated on spirit blue agar (Difco) supplemented with a 3% lipase reagent containing tributyrin as a substrate at 21°C for 72 h. Yeast and molds were enumerated on standard methods agar (BBL, Cockeysville, MD) supplemented with a 2% solution of chloramphenicol/chlorotetracycline hydrochloride (500 mg each in 100 ml of sterilized phosphate buffer) (Sigma Co., St. Louis, MO) at 25°C for 5 d. All determinations were carried out in duplicate, and counts were converted to a log scale.

Free Fatty Acids Analysis

Free fatty acids were extracted using the method of Ha and Lindsay (1990). Butylation of the FFA was achieved by adding 1 ml of 14% BF3 in butanol (Sigma Co.) to the dried ether extracts in each screw-capped test tube and boiling for 10 min. The reaction was stopped by placing the mixture in an ice-water bath. The butyl esters were recovered in 10 ml of pentane (HPLC grade, Sigma Co.). The solution was washed three times with 10 ml of 15% methanol (Sigma Co.) in distilled water. The pentane layer was concentrated to 1 ml under a gentle stream of N2 and stored in a gas...
chromatograph vial (Alltech, Deerfield, IL) at 5°C. Gas chromatographic separation of fatty acids was carried out in a Hewlett Packard series II, model 5890 gas chromatograph (Palo Alto, CA), equipped with an automatic sampler (Hewlett Packard 7673 A) and a flame ionization detector (FID). Individual fatty acids were separated using a capillary column SPB-1, 30 m × 0.25 mm × 0.25 μm (Supelco, Bellefonte, PA). Other conditions for analysis were: injector temperature at 250°C, FID temperature at 300°C, the carrier gas was hydrogen at a flow of 1.02 ml/min. The FID was supplied with hydrogen at 30 ml/min, air at 350 ml/min and nitrogen (make up gas) at 30 ml/min. All gases were ultra high purity (AGA, Specialty Gas, Maumee, OH).

The column oven temperature was programmed from 50 to 250°C at 4EC/min and from 250 to 275°C at 10°C/min after a 10-min hold at 50°C. The sample size was 1 μL. The butylated fatty acids from the cheeses were chromatographed along with a commercial standard mix containing C4–C20 fatty acids (Nu-Chek, Elysian, MN). Quantification of each free fatty acid was calculated by comparing the peak area of each fatty acid with the peak area of the internal standard, and multiplying by a method correction factor for each fatty acid as outlined by Ha and Lindsay (1990). One milliliter of nonanoic acid (0.0010 g/ml in diethyl ether) was used as internal standard (Sigma Co., St. Louis, MO). Cheese extractions were carried out in triplicate. Each extraction was injected twice in the GC and results were averaged. Samples were analyzed at 15, 60, 120, and 180 d of age.

Casein Hydrolysis

Alkaline electrophoresis. Caseins were separated using urea alkaline gel electrophoresis based on the method by Andrews (1983). A portion of the ground cheese, weighing approximately 35 g, was sampled at 15, 30, 60, 90, 120, and 180 d of ripening and was freeze-dried at −50°C with a vacuum of 400 mTorr. All reagents used in this section were electrophoresis grade (Fisher Scientific, Fair Lawn, NJ), unless indicated. One half gram of freeze-dried cheese was blended with 100 ml of 2% sodium citrate (Sigma Co.) in a commercial blender (Waring, model 31BL92 New Hartford, CT) at high speed for 3 min. The suspension was centrifuged (Beckman J2-HS, Arlington Heights, IL) at 2000 × g for 20 min at 4°C. The solidified fat was removed and the supernatant was filtered through Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, England). Thirty milliliters of the filtrate was mixed with 10 ml of 48% TCA. The mixture was allowed to sit for 30 min and filtered through a Whatman No. 42 filter paper (Bogenrief and Olson, 1995). The 12% TCA nonprotein soluble nitrogen was measured with the Kjeldahl method (AOAC, 1995). Samples were determined in duplicate, and results were averaged.

Sensory Analysis

The panel for the sensory analysis was composed of 15 trained panelists from the Wisconsin Center for Dairy Research and the Department of Food Science. Cheeses were evaluated at 60 and 180 d of age. The samples were cut into individual pieces of approximately 2 × 2 × 4 cm and tempered for 2 h at 21°C. The samples were presented to the panelists on uncoated white paper plates, each sample coded with a random three-digit number. The panelists were asked to evaluate the cheeses in terms of cheese flavor intensity, bitterness, acidity, off flavor and firmness characteristics in a five-point linear semistructured quantitative descriptive ballot (Kasprzak et al., 1994). Higher values indicate more intense flavors or stronger attributes.

Textural Analyses

Cylindrical samples of cheese were cut using a 20-mm diameter cork bore. Both ends were trimmed so the final height was 20 mm. Samples were placed in sealable plastic bags and tempered at 5°C for 1.5 h before being analyzed (Bryant et al., 1995). The texture profile analysis (TPA) was performed with a TA-XT2
Texture Analyzer (Texture Technologies Corp., Scarsdale, NY). A 50-mm diameter flat plate probe was used to compress the cheeses in two consecutive passes, and the forces required to compress the cheeses were plotted in a force × time graph. The area underneath each curve was calculated using the XT-RA software. The probe and the base were coated with mineral oil (Fisher Scientific) to decrease friction. The test was performed at 60% strain, the crosshead speed was 5 mm/s, and the chart speed was 10 mm/s. Cheese samples were analyzed in triplicate and results were averaged. The TPA chart speed was 10 mm/s. Cheese samples were analyzed in triplicate and results were averaged. The TPA hardness and chewiness were determined at 15, 30, 60, 90, 120, and 180 d of age, but there was no significant difference between the two types of cheeses. The yeast and lipolytic organisms at the end of the ripening period (180 d) were about 3.0 log_{10} cfu/g of cheese. No coliforms were detected throughout the ripening time in any of the cheeses. (Data not shown.)

### Statistical Analysis

Results were analyzed using the SAS system version 6.12 (SAS Institute, 1996). Data were analyzed using a split-plot design with milk type as the main effect and cheese age as the secondary effect. Significant differences among mean values were determined by least significant difference at P < 0.05. The cheese-making trials consisted of duplicate vats of each type of cheese on two different days of production. Analyses of each vat were performed in duplicate or triplicate, as stated in the analytical methods.

### RESULTS AND DISCUSSION

#### Cheese Composition

The chemical composition of cheeses at 15 d is shown in Table 2. The milk batches were standardized to obtain similar composition, based on the percentage fat. The bovine/ovine milk cheese had a lower moisture and higher protein content than the bovine milk cheese. It has been reported that ovine milk produces a firmer coagulum than bovine milk (Anifantakis, 1986). Raynal and Remeuf (2000) reported that whey drainage is controlled by curd shrinkage, and high gel strength could enhance gel shrinkage that expels whey from the protein network. Fat retention in the bovine/ovine milk cheese was also slightly less than the bovine milk cheese, as evidenced by the fat in the DM of 31.5 versus 32.5%, respectively.

### Microbial Analyses

The microbial populations did not differ significantly between the bovine milk cheeses and the bovine/ovine milk cheeses. The lactobacilli population increased between the bovine milk cheeses and the bovine/ovine cheeses during the ripening process. The lower concentrations of FFA in the cheeses produced in this study may have been the result of the pasteurization of the milks and the starter organism having limited lipolytic activity. The populations of lipolytic organisms were not significantly different between the two types of cheeses. The yeast and lipolytic organisms at the end of the ripening period (180 d) were about 3.0 log_{10} cfu/g of cheese. No coliforms were detected throughout the ripening time in any of the cheeses. (Data not shown.)

### Free Fatty Acids

The concentrations of total FFA did not increase significantly during the ripening time. Previous researchers (Macedo and Malcata, 1996; Najera et al., 1994) have reported significant increases in FFA in ovine milk cheeses during the ripening process. The lower concentrations of FFA in the cheeses produced in this study may have been the result of the pasteurization of the milks and the starter organism having limited lipolytic activity. The populations of lipolytic organisms were not significantly different between the cheeses. However there were higher amounts of caprylic (C8:0) and capric (C10:0) in the bovine/ovine milk cheeses (Table 3). These results were similar to those obtained by Gomez et al. (1987). They compared the free fatty acid profiles of different varieties of cheeses made with milk of different species and found that cheeses made either with ovine or caprine milk had higher amounts of short chain fatty acids.

### Table 2

<table>
<thead>
<tr>
<th>Component</th>
<th>Bovine milk</th>
<th>Bovine/ovine milk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>pH</td>
<td>5.36 ± 0.02</td>
<td>5.35 ± 0.01</td>
</tr>
<tr>
<td>Moisture, %</td>
<td>49.82 ± 3.28</td>
<td>46.78 ± 1.26</td>
</tr>
<tr>
<td>Fat, %</td>
<td>16.30 ± 1.81</td>
<td>16.74 ± 0.94</td>
</tr>
<tr>
<td>Protein, %</td>
<td>25.31 ± 0.68</td>
<td>28.31 ± 1.81</td>
</tr>
<tr>
<td>Salt, %</td>
<td>2.01 ± 0.52</td>
<td>1.99 ± 0.32</td>
</tr>
<tr>
<td>S/M, %</td>
<td>4.05 ± 0.76</td>
<td>4.25 ± 0.58</td>
</tr>
</tbody>
</table>

1Mean of duplicate determinations of four different batches of cheese.

2All components are presented as a percentage (wt/wt).

3Ratio of salt to moisture in cheese.

### Table 3

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Bovine milk</th>
<th>Bovine/ovine milk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Butyric (C4:0)</td>
<td>88.22 ± 59.97</td>
<td>70.44 ± 25.02</td>
</tr>
<tr>
<td>Caproic (C6:0)</td>
<td>38.77 ± 32.13</td>
<td>41.66 ± 15.26</td>
</tr>
<tr>
<td>Caprylic (C8:0)</td>
<td>24.30 ± 11.33</td>
<td>43.43 ± 12.48</td>
</tr>
<tr>
<td>Capric (C10:0)</td>
<td>63.20 ± 17.16</td>
<td>121.00 ± 34.95</td>
</tr>
</tbody>
</table>

abcLeast squares means within the same row without a common superscript differ (P < 0.05).
fatty acids than cheeses made with bovine milk. They attributed it to the higher amounts of capric and caprylic acid that are present in ovine and caprine milks. When free fatty acid profiles of Roquefort cheese, made with ovine milk, and Blue cheese, made with bovine milk, were compared, higher concentrations of caprylic acid were found in Roquefort than in Blue cheese (Woo et al., 1984); whereas Blue cheese had a higher concentration of butyric acid.

Casein Hydrolysis

$\alpha_{s1}$-Casein was progressively hydrolyzed throughout the ripening time and was completely hydrolyzed at 120 d of age (Figure 1). This pattern of $\alpha_{s1}$-CN hydrolysis was also observed in the ripening of other cheeses (Bogenrief and Olson, 1995; Fenelon et al., 1998; Jin and Park, 1995; Macedo and Malcata, 1996). The $\beta$-CN was not as extensively degraded as the $\alpha_{s1}$-CN. We observed that approximately 40% of the bovine $\beta$-CN was degraded within 90 d of aging, while only 16% of the $\beta$-CN in bovine/ovine cheese was hydrolyzed at 90 d (Figure 1).

Soluble Nitrogen

The TCA-soluble nitrogen of both cheeses increased from 0.10% at 15 d of age to 0.45% at 180 d of age. The activity of the starter or proteolytic enzymes released from the starter was responsible for the increased levels of TCA-soluble nitrogen (Lane and Fox, 1997). The amounts of TCA-soluble nitrogen were not significantly different between the bovine milk cheeses and the bovine/ovine milk cheeses throughout the aging period.

Sensory Analysis

Average scores for flavor, body, and firmness of the cheeses are shown in Table 4. The bovine/ovine milk cheeses were significantly firmer than the bovine milk cheeses and exhibited a greater tendency for off flavors. About 30% of the panelists identified the off flavor of the bovine/ovine milk cheese as slightly rancid, feedy, barny, or sheepy. The increased levels of caprylic ($C_{8:0}$) and capric ($C_{10:0}$) acids in the bovine/ovine milk cheeses contributed to the slight rancid flavor (Banks et al., 1997; Chavarri et al., 1999). Lopez and Lindsay (1993) reported that alkyl phenols were responsible for the barny or sheepy flavor of ovine milk. When ovine milk was blended with bovine milk for manufacture of traditional full fat ovine milk cheeses, the cheeses from mixed milk had very acceptable flavor (Krcal et al., 1982; Wendorff, 1998). The cheese flavor intensities of both cheeses were comparable throughout the aging period. Banks et al. (1997) reported that Cheddar cheese made from bovine skim milk developed a more

Table 4. Sensory attributes affected by ripening time of reduced fat Muenster-type cheeses produced from bovine milk and a mixture of bovine and ovine milk.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>60 d</th>
<th>180 d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bovine</td>
<td>Mixed</td>
</tr>
<tr>
<td>Cheese flavor intensity$^1$</td>
<td>2.87$^a$</td>
<td>2.34$^a$</td>
</tr>
<tr>
<td>Acidity$^2$</td>
<td>2.16$^a$</td>
<td>1.89$^a$</td>
</tr>
<tr>
<td>Off flavor$^3$</td>
<td>1.89$^b$</td>
<td>2.57$^a$</td>
</tr>
<tr>
<td>Firmness$^4$</td>
<td>2.43$^b$</td>
<td>3.87$^a$</td>
</tr>
</tbody>
</table>

$^a,b$Least square means (n = 60) within the same row and ripening time without a common superscript differ ($P < 0.05$). Comparisons were made for each type of milk at 60 and 180 d of age.

$^1$Sensory scores: 1 = lacks flavor, 5 = intense flavor.

$^2$Sensory scores: 1 = no acidity, 5 = sharp acid bite.

$^3$Sensory scores: 1 = no off-flavor, 5 = pronounced off flavor.

$^4$Sensory scores: 1 = very soft, 5 = very firm.
intense Cheddar flavor than that produced from ovine skim milk.

Bovine milk cheeses were softer in body and smoother in texture than bovine/ovine milk cheeses. Several researchers (Banks et al., 1997; Kalantzopoulos, 1993) reported that cheeses produced from ovine milk were firmer and more grainy than those produced from bovine milk.

Textural Analysis

TPA analysis showed that hardness and chewiness of both cheeses decreased during the aging period (Figures 2 and 3). The bovine/ovine milk cheeses were significantly harder and chewier than the bovine milk cheeses throughout the aging period; however, bovine/ovine cheeses were only chewier than bovine cheeses at 90, 120, and 180 d of aging. Creamer and Olson (1982) reported that the softening in the texture of cheese was due to the degradation of $\alpha_{s1}$-CN during ripening. However, Bogenrief and Olson (1995) later reported that hydrolysis of $\alpha_{s1}$-CN did not correlate with rheological properties at a given age of cheese. Rather, $\beta$-CN hydrolysis was more closely correlated with rheological characteristics. The difference in $\beta$-CN hydrolysis (Figure 1) between the two cheeses may have had an impact on the hardness and chewiness of the cheeses. The lower moisture in the bovine/ovine milk cheese may also have contributed to the increase hardness of the cheese.

CONCLUSIONS

The addition of ovine milk to bovine milk did result in increased amounts of caprylic and capric acids ($C_8, C_{10}$) in reduced fat Muenster-type cheeses. This had an impact on cheese flavor. In reduced fat cheeses, the off flavor noted from ovine milk may be too intense to serve as a source for additional dairy fat flavor in reduced fat bovine milk cheeses. The addition of ovine milk to bovine milk for producing reduced fat Muenster cheeses also had an impact on casein hydrolysis and firmness of the cheese. Further studies are needed to determine why bovine/ovine milk exhibited significant differences in $\beta$-CN hydrolysis and how that impacted the firmness of cheeses. It appears that the potential use of ovine milk for flavor development in reduced fat cheeses may be limited to those varieties that traditionally are made from ovine milk.

ACKNOWLEDGMENTS

We are very grateful to Yun-Fei Chen of the College of Agricultural and Life Sciences Statistical Center for assistance in statistical analysis of data. This research was supported in part by the College of Agricultural and Life Sciences, University of Wisconsin-Madison and the University of Puerto Rico-Mayagüez.

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