Differential Scanning Calorimetry of Water Buffalo and Cow Milk Fat in Mozzarella Cheese

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ABSTRACT: The thermal profiles of the fat in mozzarella cheeses made from cow milk (CM) and water buffalo milk (WBM) were obtained by differential scanning calorimetry (DSC). The DSC curves of mozzarella cheese made from WBM were distinguishable from those of CM. The curves resembled those of the corresponding milk fats and could be divided into low-, medium-, and high-temperature melting regions. The valley in the curve between the low- and medium-temperature melting regions was at 10.8°C in WBM cheese and below 10°C in CM cheese. In the WBM cheese, the area of the low-melting region was larger than the area of the medium-temperature melting region, but the two areas were equal in the CM cheeses. Mixtures of the two cheeses exhibited temperature and area values between those of the pure cheeses. Milk-fat mixtures showed similar behavior. The contrasting DSC melting profiles provide a way of distinguishing between the two mozzarella cheese types and for detecting mixtures of the two fats in mozzarella cheese.
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KEY WORDS: Cheese, cow milk, DSC, melting profile, milk fat, mozzarella, water buffalo milk.

Americans consume more mozzarella cheese than any other variety except Cheddar (1). Mozzarella in the United States is commercially manufactured from cow milk (CM), but mozzarella imported from Italy is considered a delicacy because it is manufactured from water buffalo milk (WBM), which gives the cheese its unique sensory characteristics (2). WBM mozzarella is prepared "fresh," that is, intended for consumption within 24 h, but significant amounts are imported into the United States. U.S. Customs is responsible for ensuring that imported fresh WBM mozzarella does not contain CM, which would make it subject to quota restrictions and/or tariff (3). The two methods for distinguishing the two types of cheese involve electrophoretic analysis of the proteins and gas-chromatographic evaluation of the fatty acid profiles (3).

The primary proteins in milk are α_s-, β-, and γ-caseins, which electrophoresis reveals are similar in WBM and CM; however, there are variations in protein sequences, exemplified by differences in the γ_2- and γ_3-caseins, which originate from β-casein (4). An isoelectric focusing method for distinguishing the two species (4), recently modified for miniature gels (Malin, E.L., J.J. Shieh, and B.C. Sullivan, unpublished data), is based on sequence differences in β-caseins of the two species.

There are major differences in the fat contents of WBM and CM and the cheeses made from them. CM from the United States averages 3.9% fat (5), compared with 7.2 to 7.9% for WBM from Italy (6), and the fat globules in WBM are larger and more numerous than those in CM (7). WBM fat contains more palmitic, stearic, and oleic acids than CM fat (6,7).

The melting properties of a fat can be obtained by differential scanning calorimetry (DSC). Taylor *et al.* (8) separated CM fat into low-, medium-, and high-molecular weight (MW) fractions and obtained DSC curves for each. They attributed almost all of the melting below 30°C to low-MW and unsaturated high-MW triglycerides, which melt at lower temperatures than high-MW saturated triglycerides. The low-MW triglycerides contained butyric (and some caproic) acid esterified at position 3 on the glycerol molecule, and the high-MW unsaturated triglycerides contained oleic acid at position 3. Patel and Frede (9) analyzed ghee, an Indian dairy product similar to milk fat, prepared from butter manufactured from CM and WBM. They noted differences in crystallization and melting curves obtained by DSC and in solid-fat content obtained by pulsed nuclear magnetic resonance. They divided the melting curves into low-, medium-, and high-temperature melting zones, and attributed the variations in the curves of the two species to triglyceride composition.

Although DSC is convenient for thermal analysis of cheese, DSC studies of cheese have not been reported. Caseins do not exhibit DSC melting transitions, and the fat does not have to be extracted if the sample is dried before analysis (10). The entire analysis can be completed within 2 h and can be a rapid screening method for distinguishing CM and WBM cheeses. Moreover, mixtures of WBM and CM fat also can be detected. This paper extends the DSC method to mozzarella cheeses containing CM or WBM.

MATERIALS AND METHODS

The fresh WBM mozzarella cheeses used in this study were manufactured by Mozzarella Co. (Dallas, TX) or were manufactured in Italy and supplied by the U.S. Customs Service

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Laboratory (New York, NY). The fresh CM mozzarellas were manufactured in the United States and obtained from a local retail store. Each cheese contained 25% fat, as measured by the modified Babcock method (11), and approximately 50% moisture, as measured by the forced-draft oven method (12).

A sample of CM was obtained from a farm in Philadelphia, PA, and a WBM sample was obtained from a farm in Texarkana, AR. Fat was separated from the milk by centrifugation at 16000 × g at 2°C for 35 min.

Cheese and fat specimens weighing 16 to 24 mg were removed from the interior of the refrigerated samples, chopped with a small spatula into fine pieces <1 mm on a side, placed in aluminum sample pans (Perkin-Elmer, Norwalk, CT), dried for 20 min at 120°C in a forced-draft oven, and weighed. Mixtures of cheeses or fats were prepared by weighing 4–16 mg of each specimen into each half of the sample pan and combining with the spatula to ensure homogeneity. Melting profiles were obtained with a Perkin-Elmer Model DSC-7 differential scanning calorimeter, equipped with an Intercooler-2 subambient assembly. The DSC was calibrated with an indium standard. The dried specimens were held at 50°C for 5 min to remove thermal history, cooled to –50°C at 5°C/min, held at that temperature for 15 min, and heated to 50°C at 5°C/min. Partial-areas software, provided with the instrument, was used to determine temperatures of various features on the DSC curves, areas of the melting regions, and heat of fusion.

Three replicates of each cheese, fat, and mixture were analyzed. Statistically significant differences between values were determined by Duncan's multiple range test at a 95% confidence interval (13).

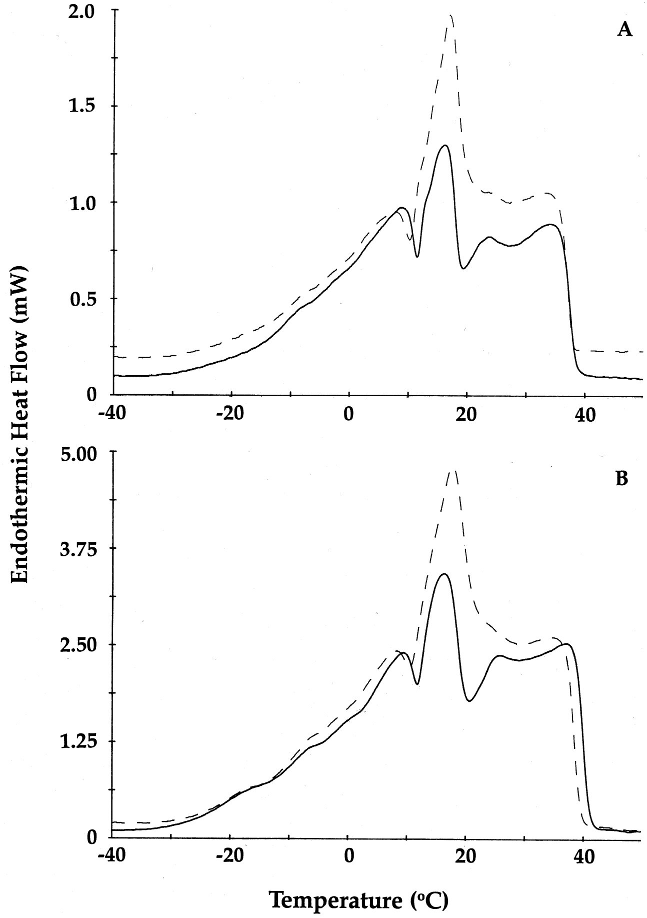

FIG. 1. (A) Differential scanning calorimetry (DSC) curves, showing milliwatts (mW) of endothermic heat flow vs. temperature, of mozzarella cheeses made from different milks. Solid line, 9.01 mg (dry weight) Italian water buffalo milk cheese; dashed line, 10.03 mg (dry weight) American cow milk cheese. (B) DSC curves of milk fats. Solid line, 17.67 mg water buffalo milk fat; dashed line, 19.13 mg cow milk fat.

RESULTS AND DISCUSSION

Figure 1A compares typical DSC melting curves of fresh mozzarella cheese made from CM and WBM. Both cheeses had low- and medium-temperature melting regions, which were separated by valleys in the curves. The fresh CM mozzarella samples had a low-temperature melting region from about –30° to 11°C, with a short peak between 7 and 8°C. A medium-temperature melting region, distinguished by a tall peak at about 17°C, preceded a high melting region, from about 21 to 38°C, consisting of a plateau with a pair of shoulders. The medium- and high-temperature melting regions were separated by either a small valley or an inflection point around 20°C. The low-temperature melting region in the WBM cheese, between about –33° and 11°C, contained a peak at about 9°C. The medium-temperature melting region was similar to that of CM, although the peak was smaller. The high melting region started with a valley or inflection point at 20°C, continued with two shoulders, and ended at 41°C. Because the determination of the exact temperature of an inflection point could be subjective, the dividing line between the medium- and high-temperature melting regions was set at 20°C. The heat of fusion values for the fat in both kinds of cheese were between 72 and 78 J/g.

Table 1 shows that the average temperature of the first valley in the curves of the CM cheeses was less than 10°C, and the low-temperature melting region contained less than 31% of the total area. In contrast, the low-temperature melting region in the WBM cheeses, made in the United States and Italy, ended at 10.8°C and represented more than 46% of the total area. The medium-temperature melting regions of the CM cheeses represented at least 33% of the total area, whereas the same region in the WBM curves was less than 28% of the area. Temperatures and areas of CM–WBM cheese mixtures were between those of the pure cheeses, as expected. The lowest level of CM cheese that could be unambiguously detected in a mixture was 25%. Mixtures containing 10% CM cheese had a first valley temperature and a medium-temperature melting region area within the standard deviation of WBM cheese.

A method of distinguishing between the cheeses of the two species is by calculating the ratio of the low-temperature melting area to the medium-temperature melting area in the DSC curve. The ratio in the CM cheese was less than 1, but

TABLE 1
DSC Data from Mixtures of Mozzarella Cheeses Manufactured from Cow Milk (CM) and Water Buffalo Milk (WBM)

	Upper temperature of melting region (°C)		Area of melting region (% of total area)		
Cheese composition	Low[a]	Medium	Low[a]	Medium[a]	Ratio[b]
		American CM cheese–American WBM cheese			
100% CM	9.7 ± .2[a]	20	30.6 ± .3[a]	33.3 ± 1.3[a]	0.92
25% WBM–75% CM	10.2 ± .1[b]	20	39.7 ± .2[b]	30.5 ± 1.4[b,d]	1.30
50% WBM–50% CM	10.4 ± .3[b,c]	20	41.9 ± .3[c]	29.7 ± 1.3[c,d]	1.41
75% WBM–25% CM	10.6 ± .2[c,d]	20	44.2 ± .3[d]	28.3 ± 1.2[c,d]	1.56
90% WBM–10% CM	10.8 ± .2[d]	20	45.3 ± .2[e]	27.8 ± 1.1[c]	1.63
100% WBM	10.8 ± .1[d]	20	46.1 ± .2[f]	27.7 ± .9[c]	1.66
		American CM cheese–Italian WBM cheese			
100% CM	8.9 ± .2[a]	20	30.6 ± .4[a]	36.0 ± .9[a]	0.85
25% WBM–75% CM	10.0 ± .1[b]	20	39.4 ± .2[b]	30.9 ± 1.3[b,d]	1.28
50% WBM–50% CM	10.4 ± .2[c]	20	41.8 ± .2[c]	29.8 ± 1.5[c,d]	1.40
75% WBM–25% CM	10.4 ± .1[c]	20	44.8 ± .1[d]	28.9 ± 1.2[c,d]	1.56
90% WBM–10% CM	10.7 ± .2[d]	20	46.2 ± .2[e]	28.3 ± 1.4[c]	1.63
100% WBM	10.8 ± .1[d]	20	46.7 ± .2[f]	27.5 ± 1.1[c]	1.70

[a]Mean value ± standard deviation for three replicates. DSC, differential scanning calorimetry.
[b]Ratio of low-temperature melting area to medium-temperature melting area. Values in each group bearing different superscripts (a–f) are significantly different.

the ratio in the WBM cheeses was greater than 1.6. Calculations of likely glyceride compositions (14) indicate that WBM fat contains more low-MW and unsaturated high-MW triglycerides than CM fat, which could account for the differences in melting characteristics. These differences are enough to distinguish WBM from CM mozzarella by DSC.

The DSC curves of WBM and CM fats extracted from milk resembled those of the corresponding cheeses (Fig. 1B) and were similar to those of WBM ghee (9) and CM mozzarella (10). Table 2 shows the upper temperatures and the areas of the low- and medium-temperature melting regions in WBM and CM fats, and in mixtures of the two. The WBM fat had a larger high-temperature melting region than the cheeses made from WBM, resulting in smaller areas in the other melting regions. The upper temperature of the low-temperature melting region was 0.8°C higher than those of the cheeses made from 100% WBM, resulting in a larger ratio of low- to medium-temperature melting areas. The ratios for the four fat mixtures fell between those of the pure fats as expected. A mixture containing 25% CM fat could be differentiated from 100% WBM fat, but a mixture containing 10% CM fat could not. The DSC curves of CM and WBM fats were similar to those of mozzarella cheeses made from them but were distinguishable from each other. All fat samples had heat of fusion values between 74 and 76 J/g.

WBM and CM fats can be distinguished in mozzarella cheese from the differences in their DSC melting profiles. The ratio of the areas of the low- and medium-temperature melting regions can be used to indicate whether cheese purported to be made from WBM contains at least 25% CM. The DSC procedure is also a rapid and accurate method for determining if WBM mozzarella has been mislabeled.

TABLE 2
DSC Data from Mixtures of CM and WBM Fats

	Upper temperature of melting region (°C)		Area of melting region (% of total area)		
Fat composition	Low[a]	Medium	Low[a]	Medium[a]	Ratio[b]
100% CM	9.2 ± .1[a]	20	33.7 ± .4[a]	32.2 ± .4[a]	1.05
25% WBM–75% CM	9.3 ± .4[a]	20	34.2 ± .3[b]	30.9 ± .8[b]	1.11
50% WBM–50% CM	9.6 ± .4[a]	20	35.8 ± .2[c]	27.5 ± .5[c]	1.30
75% WBM–25% CM	10.8 ± .2[b]	20	38.6 ± .2[d]	23.3 ± .4[d]	1.66
90% WBM–10% CM	11.4 ± .1[c]	20	40.9 ± .2[e]	20.1 ± .6[e]	2.03
100% WBM	11.6 ± .3[c]	20	41.3 ± .1[e]	19.1 ± .4[f]	2.16

[a]Mean value ± standard deviation for three replicates.
[b]Ratio of low-temperature melting area to medium-temperature melting area. Values in each group bearing different superscripts (a–f) are significantly different. See Table 1 for abbreviations.

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Mention of brand or firm names does not constitute an endorsement by the USDA over others of a similar nature not mentioned.

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