

Characterization of Natural and Imitation Mozzarella Cheeses by Differential Scanning Calorimetry

ABSTRACT

Differential scanning calorimetry was used to distinguish natural Mozzarella cheese from imitation Mozzarella made with calcium caseinate. The enthalpy of the milk fat melting transition at 18°C decreased with increasing caseinate concentration. Scanning electron microscopy studies revealed an agglomeration of lipids in the imitation samples, whereas the natural cheese had a uniform dispersion of fat globules. The addition of the caseinate apparently affected the crystallization of the fat, leading to the enthalpy reduction. Electrophoresis and atomic absorption procedures did not differentiate between natural and imitation samples.

INTRODUCTION

There is a steadily growing market in the United States for Italian cheeses such as Mozzarella. Over 1.2 billion lb of Mozzarella were produced in the US in 1986, an increase of 11% over the 1985 figure (1). The US government purchases large quantities of low moisture, part skim Mozzarella for use in the national school lunch program and other food donation programs. The specifications for manufacture do not allow for the use of caseinates, which, if present in the milk at a concentration of 1%, would increase the yield by up to 24% (7). Because of the potential for substituting imitation Mozzarella made with caseinate, an inves-

tigation into the physical properties of natural and imitation Mozzarella was conducted.

MATERIALS AND METHODS

Low moisture part skim Mozzarella cheeses were made with locally obtained fresh raw milk in accordance with procedures described by Kosikowski (8). These samples were identified as natural Mozzarella containing 0% calcium caseinate. Calcium caseinate powder (New Zealand Milk Products, Inc., Petaluma, CA¹) was stirred into the milk of the imitation cheeses to a concentration of 1 or 2% by weight. One imitation cheese was made with sodium caseinate from the same supplier and contained no milk. Samples were vacuum-packaged and stored at 4°C.

Proteins were extracted from the cheese by solubilizing in Tris-EDTA buffer, adding SDS and dithiothreitol, and centrifuging (5). Three fractions were obtained: a supernate (S), a pellet (P), and a fat pellicle (F). The latter two fractions were homogenized in the buffer and centrifuged again to obtain a pellet supernate (PS) and a fat pellicle supernate (FS). The fractions were lyophilized and examined by PAGE using the discontinuous system of Laemmli (9), as modified by Basch et al. (4).

Samples were prepared for calcium determinations by heating 1 g of finely chopped cheese at 560°C for 16 h, dissolving the ash in 2 ml of 50% HCl, and diluting to a final HCl concentration of 1%. Calcium levels were then measured with a Perkin-Elmer 1100B atomic absorption spectrometer (Perkin-Elmer Corp., Norwalk, CT). Fat determinations were made using the Babcock method (2).

Thermal behavior was examined by differential scanning calorimetry (DSC) on a Perkin-Elmer DSC-2. Samples weighing 2 to 6 mg were sealed in volatile sample pans, placed in

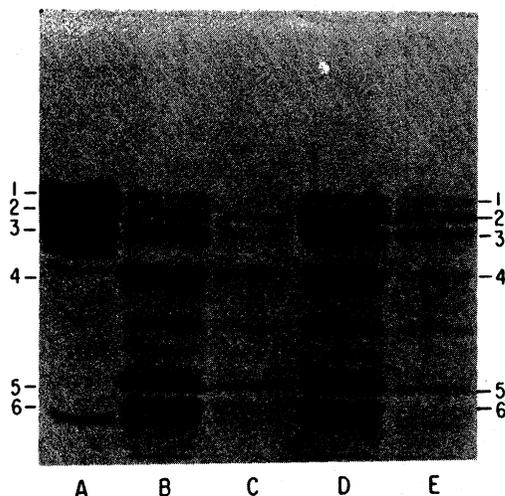


Figure 1. The PAGE of sodium caseinate powder and natural Mozzarella fractions supernate, fat pellicle supernate, pellet supernate, and pellet in lanes A to E, respectively. Bands: 1) α_{s2} -casein; 2) α_{s1} -casein; 3) β -casein; 3A) κ -casein; 4) γ_1 -casein; 5) *para*- κ -casein; 6) γ_2 -casein, and γ_3 -casein.

the instrument, and heated at 10°C/min with nitrogen as the purge gas. Temperature-treated samples were held at 60°C for 5 min, cooled at 10°C/min to -40°C, and held at that temperature for 5 min prior to heating. Samples that were not temperature treated were heated from 5 to 25°C.

Samples were prepared for scanning electron microscopy (SEM) by procedures described by Kalab (6). A sliver taken from the center of each cheese was minced into 1- to 2-mm cubes and fixed with 3.5% glutaraldehyde in .05% sodium cacodylate (pH 6.6) at 4°C overnight. After three rinses with the cacodylate, the sample was postfixated with 2% osmium tetroxide in cacodylate at 25°C for 2 h. After three more cacodylate rinses, the sample was dehydrated by a graded ethanol series, freeze-fractured, critical-point dried, mounted, given a sputter coat of 20 nm of gold, and examined with a JEOL JSM-840A SEM operating at 10 kV.

RESULTS AND DISCUSSION

Electrophoresis

The proteins of natural and imitation low moisture part skim Mozzarella cheeses were

examined electrophoretically. The typical breakdown of major caseins was observed: α_{s2} - and α_{s1} -caseins into large fragmentary proteins, β -casein into γ_2 - and γ_3 -caseins, and κ -casein into *para*- κ -casein and macropeptides. Natural Mozzarella (Figure 1) and Mozzarella made with 100% sodium caseinate (Figure 2) were similar, but the α -casein bands were weaker in the imitation cheese. When cheeses containing 0, 1, and 2% calcium caseinate were compared (Figure 3), the bands were identical. Electrophoresis is therefore not sensitive enough to detect small amounts of caseinate in Mozzarella.

Calcium Determination

The calcium content of the cheeses ranged from 454 to 555 mg/100 g cheese (Table 1). The relatively narrow range of values precludes the use of calcium concentration as a diagnostic method for differentiating natural and imitation Mozzarellas.

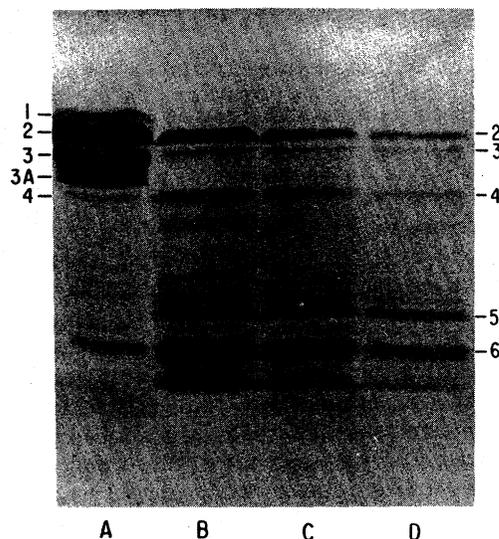


Figure 2. The PAGE of sodium caseinate powder and imitation Mozzarella fractions S, FS, and PS in lanes A to D, respectively. Bands: 1) α_{s2} -casein; 2) α_{s1} -casein; 3) β -casein; 3A) κ -casein; 4) γ_1 -casein; 5) *para*- κ -casein; 6) γ_2 -casein, and γ_3 -casein. Cheese made with sodium caseinate instead of milk.

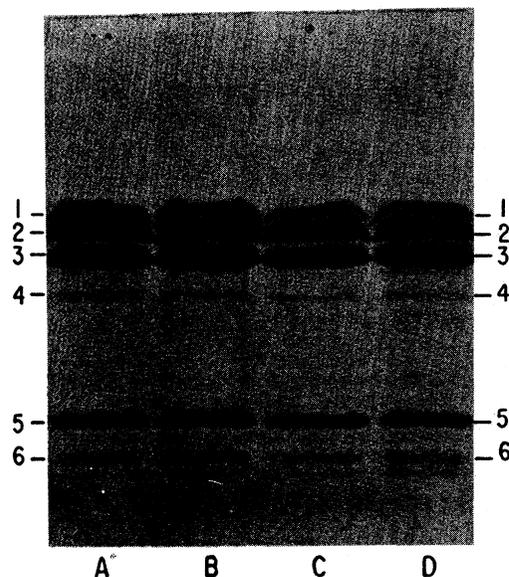


Figure 3. The PAGE of Mozzarella made with 0, 1, 1, and 2% calcium caseinate in lanes A to D, respectively. Bands: 1) α_{s2} -casein; 2) α_{s1} -casein; 3) β -casein; 3A) κ -casein; 4) γ_1 -casein; 5) *para*- κ -casein; 6) γ_2 -casein, and γ_3 -casein.

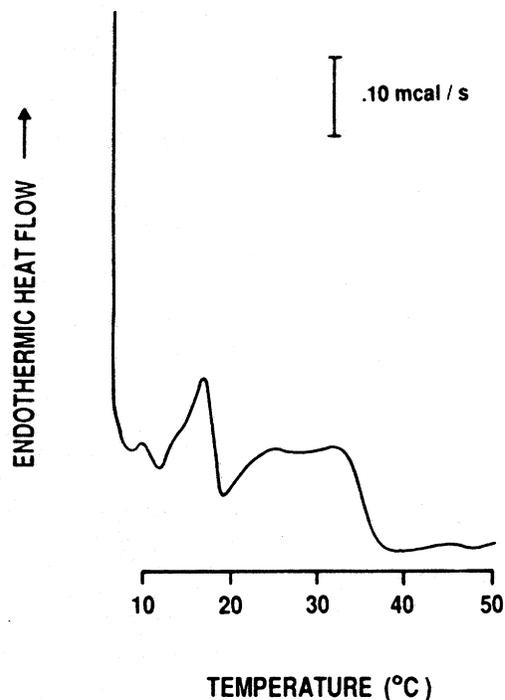


Figure 4. Differential scanning calorimetry curve of 7.4 mg of temperature-treated Mozzarella.

Differential Scanning Calorimetry

The DSC curves can clearly show the thermal behavior of the water and fat in cheese. When a sample containing fat is held above its melting point in the DSC, the thermal history of the fat is erased. Cooling quickly and holding at a temperature below the point where melting begins will allow the fat to crystallize in a more uniform manner (3). Treating a Mozzarella sample in this way produces a DSC curve such as the one in Figure 4. Endothermic transitions corresponding to melting of milk fat

triglycerides are found at temperatures above the 0°C water peak. All of the natural and imitation Mozzarellas had similar DSC curves after undergoing this temperature treatment.

Different results are obtained if the samples instead are removed from 4°C storage, placed in the DSC at 5°C and scanned immediately from 5 to 25°C. In this case, the thermal transition around 16 to 18°C varies according to the caseinate concentration (Figure 5). These differences can be quantitated by comparing the enthalpies of these peaks. The enthalpy (ΔH) of a transition is equal to the peak area in mcal divided by the weight of the sample in milligrams. To account for differences in fat content, the weight was considered to be that of the fat in the sample or percentage of fat times milligrams of sample. Table 2 shows the variation in ΔH of the 18°C transition among Mozzarellas with no temperature treatment. At least five replicate analyses were performed on each cheese to minimize the role of variability in sample composition. The transition temperature

TABLE 1. Calcium contents of natural and imitation Mozzarellas from four sample preparation runs.

Calcium caseinate (%)	Run 1	Run 2	Run 3	Run 4
	— (mg calcium/100 g cheese) —			
0	507	555	484	508
1	505	463	464	539
2	479	460	454	474

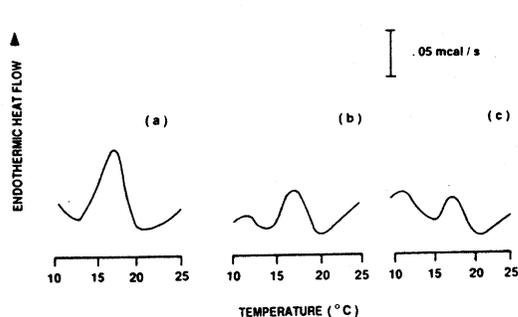


Figure 5. Differential scanning calorimetry curves, without temperature treatment, of Mozzarellas made with 0, 1, and 2% calcium caseinate, respectively. Each sample weighed 4.6 mg.

of the lipids does not change with caseinate concentration, although the freezing point of the water in the cheese decreases slightly when caseinate is present.

Microscopy

The DSC results can be explained by comparing the microstructures of natural and imitation Mozzarellas. Fat globules are uniformly dispersed throughout the protein network of natural Mozzarella, but are flattened, randomly dispersed, and often agglomerated into larger bodies in Mozzarella containing 2% caseinate (Figure 6). The matrix in the 2% caseinate cheese has zones in which there are large areas of protein without lipid and other areas where fat is plentiful. The 1% caseinate Mozzarella shows a mixture of the effects observed in the

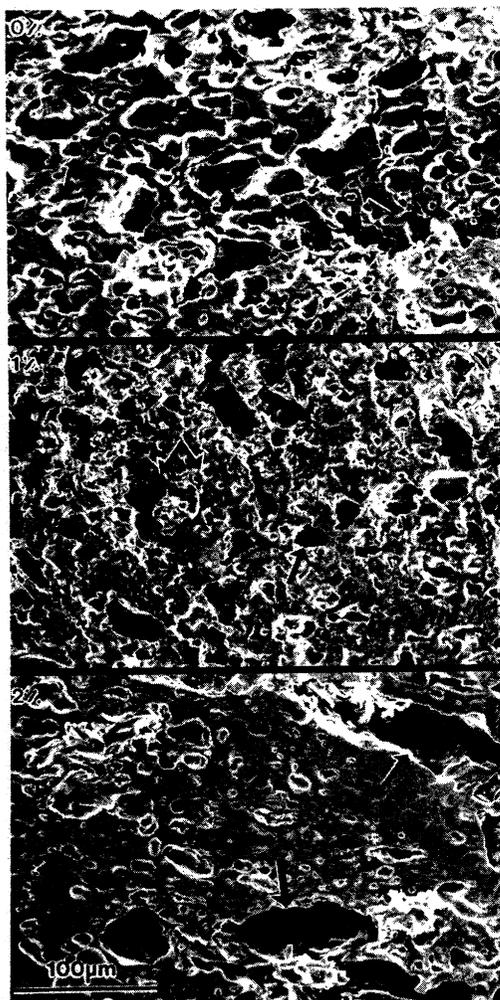


Figure 6. Scanning electron micrographs of Mozzarellas made with 0, 1, and 2% calcium caseinate, respectively. Arrows indicate sites occupied by fat globules.

TABLE 2. Enthalpies of 18°C transitions in natural and imitation Mozzarellas from two sample preparation runs.

Calcium caseinate (%)	Run 1 (n = 5)		Run 2 (n = 7)	
	\bar{X}	SD	\bar{X}	SD
0	2.44	.51	2.69	.60
1	1.12	.38	1.09	.29
2	.59	.09	.55	.07

natural and 2% caseinate cheeses. The change in the characteristics in the fat globules in imitation Mozzarella has been noted previously (10). Temperature treatment of either type of cheese results in redistribution of the globules, causing both types to contain a uniform dispersion of fat, leading to similar DSC curves. By scanning without temperature treatment, the dispersion of fat globules is unaltered and differences in thermal behavior can be observed. It

is possible that the emulsifying properties of the caseinate prevent some of the fat from crystallizing. Therefore, less fat will melt when the cheese is heated, resulting in a smaller melting transition and a lower ΔH .

CONCLUSIONS

The DSC is a rapid and sensitive technique for distinguishing natural and imitation Mozzarella cheese when the enthalpy of the 18°C milk fat melting transition is examined. The differential scanning calorimeter is a fairly common instrument in food science laboratories, and the procedure for analyzing caseinate in cheese takes less than 10 min. The SEM also produces clear results, but the process consumes several days. Electrophoresis and calcium determinations could not differentiate natural and imitation Mozzarellas when the caseinate concentration was 1 to 2%.

ACKNOWLEDGMENTS

The authors thank Ray Kwoczak for technical assistance and Steve Pollard of USDA's Agricultural Marketing Service for helpful discussions.

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