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# Detection of mislabeled butter and cheese by differential scanning calorimetry and rheology

U.S. GOVERNMENT action agencies such as the Customs Service (Department of Treasury), the Food and Drug Administration (Department of Health and Human Services), and the Agricultural Marketing Service (Department of Agriculture) occasionally deal with dairy products that have been misrepresented on their labels. Importers or manufacturers may attempt to substitute imitation or less expensive products for genuine butter or cheese. Methods for detecting mislabeling, such as gas chromatographic (GC) analysis of fatty acids and gel electrophoresis of proteins, have been used for years. However, some suspect samples require other techniques that deal with physical properties.

Differential scanning calorimetry (DSC) provides information on the enthalpy ($\Delta H$) and melting point (MP) of a sample or a constituent of a sample. The melting curves of many products, such as milk fat, have characteristic shapes that differentiate them from other products. DSC is a particularly convenient method for examining fat in butter and cheese since the only other major ingredients are casein, which does not exhibit DSC melting transitions, and water, which can be evaporated.

Empirical, imitative, and fundamental tests for measuring rheological properties of butter and cheese have been used for many years, since texture is an important characteristic of these products. Texture profile analysis of foods, an imitative test that mimics chewing, was developed in the 1960s. Fundamental data such as stress, strain, viscosity, and elastic and viscous moduli are obtained by using small amplitude oscillatory shear on a solid sample held between parallel plates. Cheeses with notably different textures produce contrasting rheological results, which are more objective than human graders.[1] This paper describes the use of DSC and rheological methods to determine if butter and cheese samples have been properly labeled.

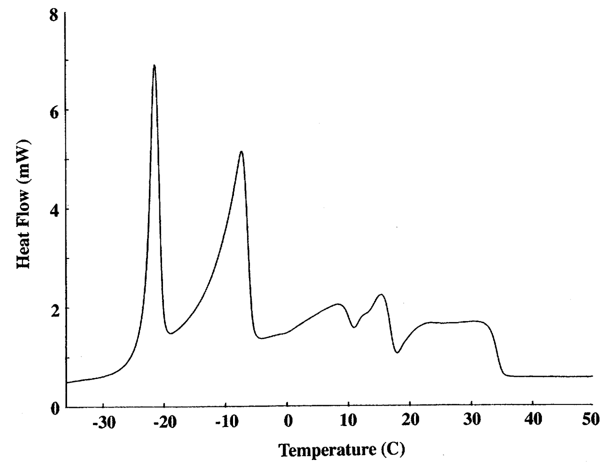


**Figure 1** *DSC heating curve of 4.7 mg of dried salted butter.*

*Butter–vegetable oil spread*

Fats are tempered in the DSC before scanning to erase thermal history and to allow uniform crystallization. For analysis, samples containing milk fat are weighed into volatile sample pans, placed in a DSC7 (**Perkin-Elmer Corp.,** Norwalk, CT), heated at 50 °C for 5 min to erase thermal history, cooled at 5 °C/min to –50 °C, held at that temperature for 15 min, and finally scanned at 5 °C/min to generate a heating curve.[2] Such a procedure is used to distinguish butter from breakfast spreads containing butter and vegetable oil. A DSC heating curve of a typical salted butter is shown in *Figure 1*.

The thermal transitions below –20 and 0 °C are both due to melting ice. When salt water is cooled below

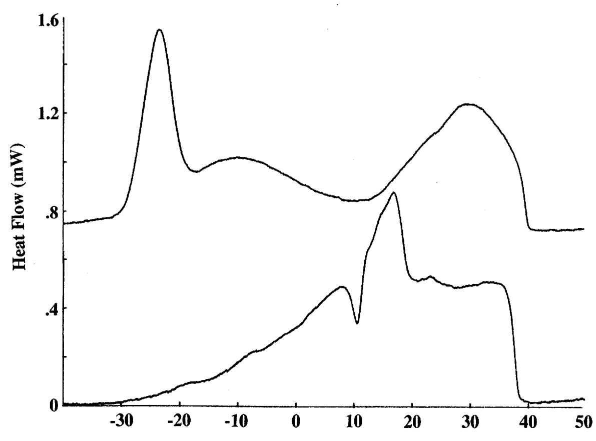


**Figure 2** *DSC heating curves of 3.4 mg of dried corn oil margarine (top) and 3.4 mg of dried butter (bottom).*

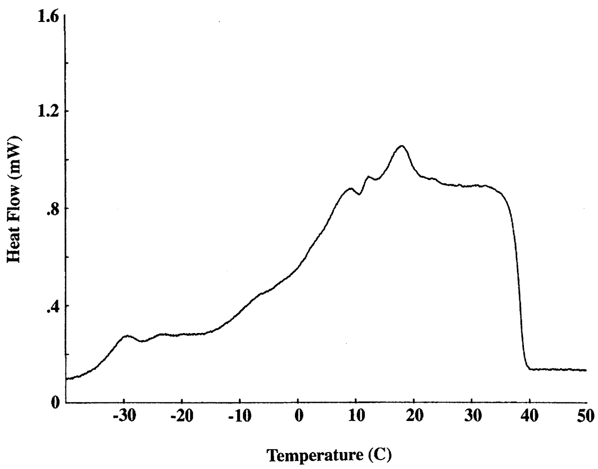


**Figure 3** *DSC heating curve of 6.2 mg of dried spread containing 65% butter and 35% corn oil margarine.*

–20 °C, the solute becomes concentrated, allowing the pure water to freeze. Eventually, the salt water freezes as well, leading to two melting peaks upon heating in the DSC. These transitions obscure those of melting fat; to prevent this, the sample is weighed into DSC sample pans and dehydrated in an oven for 20 min at 105 °C before analysis. *Figure 2* compares the characteristic melting profile of butter without interference from water with a dehydrated sample of corn oil margarine. A spread containing 65% butter and 35% corn oil margarine (*Figure 3*) is easily distinguished from butter by the appearance of the melting curve. The presence of foreign fats in butter can be detected by this method.

*Recombined butter*

Recombined butter is manufactured in developing countries where the supply of fresh milk cannot keep up

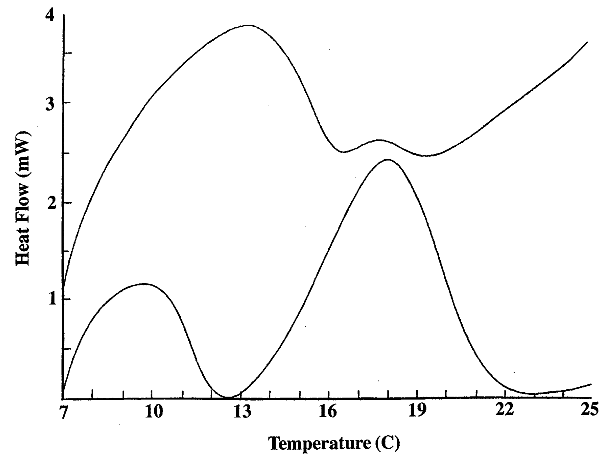


**Figure 4** *DSC curves of 13.5 mg of recombined butter (top) and 15.1 mg of natural butter (bottom), scanned from 5 to 25 °C only.*

with demand.[3] It is made from anhydrous milk fat or butter oil, water, skim milk powder, salt, and lecithin, all of which can be conveniently stored without refrigeration in a hot-weather climate.[4] In some instances, a manufacturer may find it economical to substitute recombined butter for natural butter, which by law[5] consists of churned cream and salt only. Differentiation between the two products is difficult and time consuming, involving nuclear magnetic resonance (NMR) analysis of an extracted sample to determine if lecithin is present. DSC analysis, however, reveals the effect of the dispersion of fat on its melting properties and is used to distinguish between the products within 20 min.[6]

DSC scans of natural and recombined butters appear similar when the samples have been tempered. Different results are obtained when the samples are removed from the refrigerator, weighed, placed in the DSC at 5 °C, and heated immediately. The peak around 17–20 °C in natural butter has a $\Delta H$ of 11.1 ± 0.47 J/g fat, but the transition in the same temperature range in recombined butter has a $\Delta H$ of 0.15 ± 0.03 J/g fat (*Figure 4*). Between 6 and 30% of the fat in butter consists of intact fat globules; anhydrous butter oil, which is at least 99.8% milk fat, contains no globules.[7] The fat phase in recombined butter is therefore more homogeneous than in natural butter and completes its melting several degrees earlier.

*Natural and imitation mozzarella*

In the U.S., mozzarella cheese is made from cow milk and enzymes. Adding calcium caseinate powder to the milk to a concentration of just 1% would increase the cheesemaker's yield by 24%,[8] but the product would have to be labeled as imitation. A manufacturer may be tempted to substitute the imitation cheese for

Table 1

**Enthalpies of 17–20 °C transitions in mozzarella cheeses from two sample preparation runs**

| Calcium caseinate (%) | ΔH (J/g fat) Run 1 | Run2 |
|---|---|---|
| 0 | 10.21 | 11.25 |
| 1 | 4.69 | 4.56 |
| 2 | 2.47 | 2.30 |

the genuine mozzarella, particularly since the two products are compositionally similar. However, they are distinguishable by using the same DSC method for differentiating natural from recombined butter.[9] For this purpose, duplicate sets of mozzarella cheeses containing 0, 1, and 2% calcium caseinate were prepared and scanned from 5 °C; the differences in the enthalpies in the 17–20 °C peaks are shown in *Table 1*. Calcium caseinate acts as an emulsifier, apparently preventing some of the fat from crystallizing during refrigerator storage and resulting in a smaller thermal transition in the 17–20 °C range when the sample is heated.

Texture profile analyses indicate that calcium caseinate also causes mozzarella to be firmer. A model 4201 universal testing machine (**Instron, Inc.,** Canton, MA) is used to determine hardness and cohesiveness of cylindrical samples approx. 14 mm in diameter and 14 mm high. The specimen is placed on a plate and compressed by 75% by an upper plate traveling 100 mm/min. The upper plate is returned to its original position and the cycle is repeated. The peak force in newtons (N) during the first "bite" is hardness, and the ratio of the force area during the second bite to that during the first bite is cohesiveness.[1] Part-skim mozzarellas refrigerated for one week and containing 0–1% calcium caseinate exhibit hardness values in the 60–70 N range, but cheeses containing 2% calcium caseinate have values above 80 N. Cohesiveness is 0.50–0.60 in the 2% calcium caseinate samples and 0.40–0.47 in the others.

### *Cow and buffalo milk mozzarella*

In Italy, mozzarella cheese has traditionally been made with water buffalo milk (WBM). Imported Italian-manufactured mozzarella must not contain cow milk (CM), which would make it subject to quota restrictions and/or tariff.[10] This fact, and the premium price that genuine imported Italian mozzarella commands, provide an incentive for mislabeling CM mozzarella as WBM mozzarella.[11] DSC provides an effective means of screening a cheese sample for fat melting characteristics, and can be used in conjunction with electrophoresis and GC to detect the presence of milk from different species. The differences in fat melting between CM and WBM mozzarellas are examined by DSC using partial areas software;[12] a comparison is

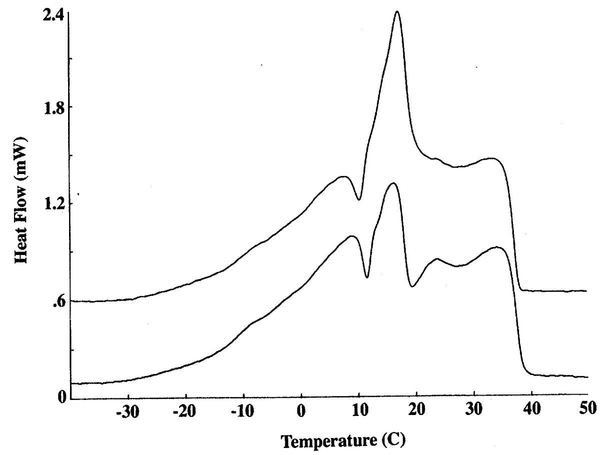


**Figure 5** *DSC heating curves of 9.0 mg of dried cow milk mozzarella cheese (top) and 10.0 mg of dried water buffalo milk mozzarella cheese (bottom).*

Table 2

**Rheological data for Italian cheese varieties**

| Variety | Activation energy (kcal/mol) | Elastic modulus (N/cm²) | Viscous modulus (N/cm²) |
|---|---|---|---|
| Parmesan | 37.40 | 12.1 | 4.1 |
| Romano | 34.44 | 9.0 | 2.6 |
| Provolone | 16.37 | 6.2 | 2.0 |
| Part-skim mozzarella | 16.75 | 3.8 | 1.4 |

shown in *Figure 5*. The lower melting region in the CM cheese, which is between –30 and 10 °C, contains 30.6 ± 0.4% of the total ΔH of the fat. The medium melting region, between 10 and 20 °C, contained 36.0 ± 0.9% of the enthalpy, a ratio of 0.85. In the WBM mozzarella, the lower and medium melting regions contain 46.7 ± 0.2% and 27.5 ± 1.1% of the enthalpy, respectively, for a ratio of 1.70. Mixtures of the two milks result in predictable ratios in between. The fat globules in WBM are larger and more numerous than those in CM, and the fat itself is more saturated,[13] accounting for the different melting profiles.

### *Italian cheese varieties*

Dynamic oscillatory shear measurements are used in conjunction with other results to determine if a cheese is labeled as the wrong variety. For detection of mislabeling, an RDA-700 dynamic analyzer (**Rheometric Scientific,** Piscataway, NJ) in a parallel plate configuration may be used to measure viscoelastic properties of sample disks with a diameter of 2.5 cm and a height of 4 mm, as described below. Elastic modulus (G′), which is a measure of the energy stored per strain cycle in a sample, and viscous modules (G″), which is a measure of the energy lost as heat per cycle, are measured as the frequency (ω) is increased from 0 to 100 rad/sec at 0.1% strain. Complex viscosity (η*) data are then obtained at a frequency of 100 rad/sec over a range of temperatures using the equations $G^* = [(G')^2 + (G'')^2]^{0.5}$ and $\eta^* = G^*/\omega$. With each sample, a plot of log η* versus reciprocal of absolute temperature (1/T) produces a straight line, which follows the Arrhenius equation. The activation energy is calculated by multiplying the slope of the line by the gas constant (1.987 cal/K mol).[14]

Dynamic oscillatory shear results for the different Italian cheese varieties shown in *Table 2* demonstrate the different textures of the products. Gel electrophoresis data are evaluated to determine if the degree of protein breakdown affects rheological results; for example, excessive breakdown of $\alpha_{s1}$-casein would soften the cheese too much. The percentages of fat and moisture in the suspect sample are also found and are compared with those listed in the Federal Standards of Identity. From this information, an investigator can draw three conclusions: 1) The sample is actually the labeled variety, 2) the cheese is a substandard version, or 3) the sample is a different cheese variety altogether.

*Cheddar and Cheshire cheeses*

Cheshire cheese is another product that is not subject to import duty since it is made only in the U.K. and not in the U.S. An importer or foreign manufacturer may try to avoid paying duty by mislabeling as Cheshire another cheese variety, such as Cheddar. Cheddar and Cheshire are both made from cow milk and have similar compositions and melting profiles. Therefore, other techniques must be used to distinguish between them. Cheshire is a crumbly cheese that is manufactured to keep curd particles separate, whereas Cheddar is made by matting the curd particles together. Dynamic oscillatory shear provides an objective method of quantitating these textural differences. In the authors' study, it was found that approximate values of log η* for 60-week-old Cheshire decreased from 6.0 at 20 °C to 4.9 at 40 °C; the corresponding values for 60-week-old Cheddar decreased from 6.2 to 4.6. The activation energies were 20.25 kcal/mol for Cheshire and 32.75 kcal/mol for Cheddar, indicating that the body of the Cheshire broke down more easily.[15]

*Summary*

Varieties of butter and cheese have different thermal and rheological properties, which are also sensitive to changes in ingredients and processing. DSC and oscillatory shear measurements enable investigators to detect misrepresentation of these dairy products, ensuring that consumers get what they pay for.

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