Detection of Refined Animal and Vegetable Fats in Adulteration of Pure Milkfat

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The objective of this research was to evaluate the presence of dehydroxylated sterols in samples of butter adulterated with exogenous refined fat of both animal and vegetable origin. The 3,5-cholestadiene, derived from the refining treatment, was found to be an index for the addition of refined beef tallow to butter. Moreover, several dehydroxylated phytosterols could be detected in butter adulterated with vegetable oil. The proposed method seemed to be a suitable criterion for detecting adulteration of pure butter with other fats.

KEY WORDS: Dehydroxylated sterols, milkfat genuineness, refined fats.

The addition of extraneous fats to milkfat can be detected through several analytical methods. The determination of phytosterols allows the detection of small amounts of vegetable fat. The analysis of fatty acids followed by calculation of suitable ratios (1–5) is commonly used to detect extraneous animal fats, although fatty acids are strongly subject to variations due to natural factors (e.g., season and period of lactation). In recent years, these variations have increased due to the introduction of some lipidic sources (i.e., whole cottonseed, flaked soybean and calcium soaps) in cows' diets (6). Consequently, the addition of extraneous animal fats, especially of small amounts, to milkfat was hardly detectable. Different approaches were proposed to increase the limit of detection and to solve the abovementioned problems.

Several authors (7–14) have detected small amounts of beef tallow added to butter by evaluating the fatty acid composition of the monoglycerides obtained by enzymatic hydrolysis. Mariani *et al.* (15) have detected beef tallow addition by determining the cholesterol esters and the diglycerides naturally present. Recently, Precht (16,17) has carried out research on the triglyceride composition of a large number of butter samples. He has shown that the addition of 1–3 and 3–5% of vegetable and animal fats, respectively, can be detected by using suitable statistical parameters.

All these methods are only based on the natural components of fat. However, if the adulterating fat has been refined, it can also contain substances deriving from the refining processes (18–27). These substances (i.e., hydrocarbons from the dehydroxylation of sterols) could be used as an index for the addition of refined fat to untreated fat. Beef tallow, for example, is characterized by a strong flavor and light brown color and, consequently, needs to be partially or completely refined to be added to milkfat.

Therefore, research was carried out to evaluate 3,5-dehydroxylated sterols that result from refining processes. Assuming that butter is not subjected to any refining treatment, the presence of 3,5-cholestadiene and 3,5-stigmastadiene in butter may be correlated to the addition of beef tallow and vegetable oil, respectively.

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EXPERIMENTAL PROCEDURES

Materials. Analyses were carried out on the following fats: 21 genuine butter samples, 1 butter sample of unknown origin, 8 samples of butter spiked with 1–10% beef tallow, 4 refined beef tallow samples and 1 sample of butter spiked with 2% soybean oil.

Sample preparation. Following the procedure described by Mariani *et al.* (26), 350 mg of fat was dissolved in 1 mL *n*-hexane and transferred onto a glass chromatographic column (40 cm length, 15 mm i.d.) that contained 15 g of silica gel (Merck 7754; Merck, Darmstadt, Germany).

Freshly distilled *n*-hexane (Merck) was used as eluent and the first fraction (30 mL) was eliminated. The subsequent fraction (35 mL) was collected, and 10 μL of internal standard solution, containing 0.1% of *n*-dotriacontane (Sigma Chemical Co., St. Louis, MO) in *n*-hexane, was added, and the solvent phase was removed under vacuum at 40°C. The dried extract was diluted with 300 μL *n*-heptane (Merck); 1 μL of this solution was injected into a gas chromatograph.

Gas chromatographic procedure. Gas–liquid chromatography apparatus was a Carlo Erba Fractovap (model 2960; Carlo Erba, Milan, Italy) equipped with an SE52 (5% phenyl, 95% methylpolysiloxane) fused-silica capillary column (20 m length, 0.32 mm i.d., 0.12 μ film thickness). The injection technique was on-column, and the column temperature was increased at 40°C/min from 80 to 220°C, then by 4.5°C/min to 280°C (10 min). The temperature of the flame-ionization detector was 300°C. The rate of the hydrogen carrier gas was 2.5 mL/min.

The identification of dehydroxylated sterols has been performed by comparison with the gas-chromatographic retention times of standards (Sigma) on three capillary columns of different polarity—OV1 (100% methylpolysiloxane), SE52 (5% phenyl, 95% methylpolysiloxane) and OV17 (50% diphenyl, 50% methylpolysiloxane).

RESULTS AND DISCUSSION

The results from the determination of 3,5-cholestadiene in genuine butter and beef tallow samples are reported in Table 1.

More than 50% of the butter samples showed no detectable amounts of 3,5-cholestadiene, and the rest showed only very low values (0.01–0.10 ppm).

Technological treatments of cream separation did not influence the presence of dehydroxylated cholesterol in genuine butter. No differences were found in sample no. 1, obtained from whey, and sample no. 17, subjected to a decholesterolyzation process by cyclodextrines, as compared to the other samples. On the contrary, the beef tallow samples showed a high concentration of dehydroxylated cholesterol.

Table 2 shows the results from eight different butter samples spiked with different beef tallow concentrations.

TABLE 1

Concentration of 3,5-Cholestadiene in Genuine Butter and Beef Tallow Samples

Sample number	Cream treatment	Origin	3,5-Cholestadiene (ppm)
Butter			
1[a]	Centrifugation	Italy	0.01
2	Centrifugation	Italy	0.06
3	Centrifugation	Ireland	0.10
4	Gravity-separation	Italy	0.08
5	Gravity-separation	Italy	0.06
6	Centrifugation	Italy	0.02
7	Centrifugation	Italy	0.06
8	Centrifugation	Italy	0.01
Average (SD)			0.05 (0.03)
9	Centrifugation	Denmark	ND[c]
10	Gravity-Separation	Italy	ND
11	Centrifugation	The Netherlands	ND
12	Centrifugation	England	ND
13	Centrifugation	England	ND
14	Gravity-separation	Italy	ND
15	Gravity-separation	Italy	ND
16	Centrifugation	England	ND
17[b]	Centrifugation	Belgium	ND
18	Centrifugation	Germany	ND
19	Centrifugation	Italy	ND
20	Centrifugation	Italy	ND
21	Centrifugation	Italy	ND
Beef tallow			
1		Italy	24.2
2		Italy	29.0
3		Italy	21.3
4		Italy	34.1
Average (SD)			27.1 (4.9)

[a]Sample of butter obtained from whey.
[b]Sample of low-cholesterol butter.
[c]ND, not detected.

TABLE 2

Concentration of 3,5-Cholestadiene in Butter Samples Spiked with Different Amounts of Beef Tallow

Beef tallow (%)	3,5-Cholestadiene (ppm)
1	0.26
1	0.31
2	0.49
2	0.50
5	0.98
5	1.26
10	2.41
10	2.47

The butter and tallow samples used for preparing the mixtures were chosen at random.

A comparison between these values and those of genuine butter (Table 1) shows that the sample containing only 1% of beef tallow showed a 3,5-cholestadiene concentration of 0.31 ppm, which is definitely higher than the maximum value (0.10 ppm) detected in the genuine butter samples.

An example of the gas-chromatographic profiles of both genuine butter and butter spiked with different beef tallow concentrations is reported in Figure 1. The increase in beef tallow in spiked samples resulted in an increased peak corresponding to 3,5-cholestadiene. A good correlation was found between the concentration of this compound and the amount of beef tallow added to butter, as confirmed by the value r^2 (0.989) as calculated by linear regression (Fig. 2).

The equation of regression made it possible to evaluate the lowest beef tallow amount detectable by this analytical method. If all the genuine butters had undetectable concentrations of 3,5-cholestadiene, the minimum amount of beef tallow detectable in adulterated butters would be equal to the value of the intercept (0.02%). The presence of 3,5-cholestadiene, also in genuine butter, cannot be ignored. The application of the equation parameters shows that the maximum value detected (0.10 ppm) corresponds to 0.43% of added beef tallow.

A butter sample of unknown origin, characterized by a regular sterol composition and an unusual fatty acid and triglyceride composition, was also analyzed. This sample showed 2.62 ppm of 3,5-cholestadiene, which corresponds to about 11% of beef tallow addition. To validate this result, the cholesterol ester composition of this sample was determined. Results proved to be similar to those obtained in previous work (15) on butter adulterated with 10% beef tallow.

The determination of dehydroxysterols can also be applied to detect the presence of vegetable fat. Figure 3 shows the gas-chromatographic profile of a butter sample adulterated with 2% of refined soybean oil. There are

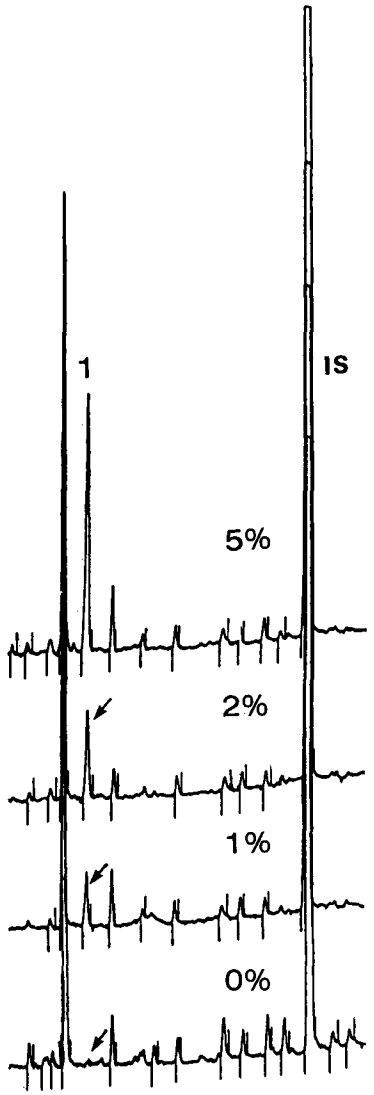

FIG. 1. The 3,5-cholestadiene region of the gas chromatograms of genuine butter (0%) and butter samples spiked with 1, 2 and 5% beef tallow. 1, 3,5-Cholestadiene; IS, internal standard.

several dehydroxylated sterols (i.e., 3,5-campestadiene, 3,5-stigmastatriene, 3,5-stigmastadiene) deriving from the phytosterols naturally present in soybean oil.

Because genuine butter does not contain phytosterols or dehydroxylated phytosterols, the analytical method may also be used to detect low concentrations of refined vegetable fat.

Moreover, analysis of dehydroxylated sterols may also allow the evaluation of vegetable oils subjected to desterolyzation. This technological process, which removes about 90% of the sterols, is responsible for the production of both dehydroxylated sterols and *trans* monoenoic acids (28).

The presence of desterolyzed oil in butter cannot be detected by traditional sterol analysis. Similarly, the determination of *trans* monoenoic acids (29) is not suitable because genuine butter contains a certain amount of these compounds.

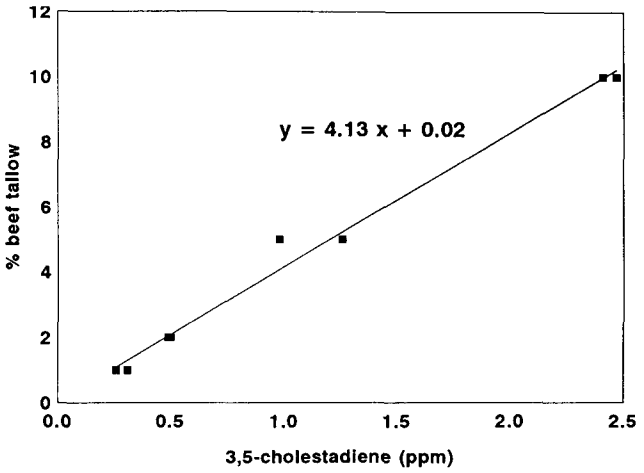

FIG. 2. Regression line between the concentration of 3,5-cholestadiene and the amount of beef tallow added to butter samples.

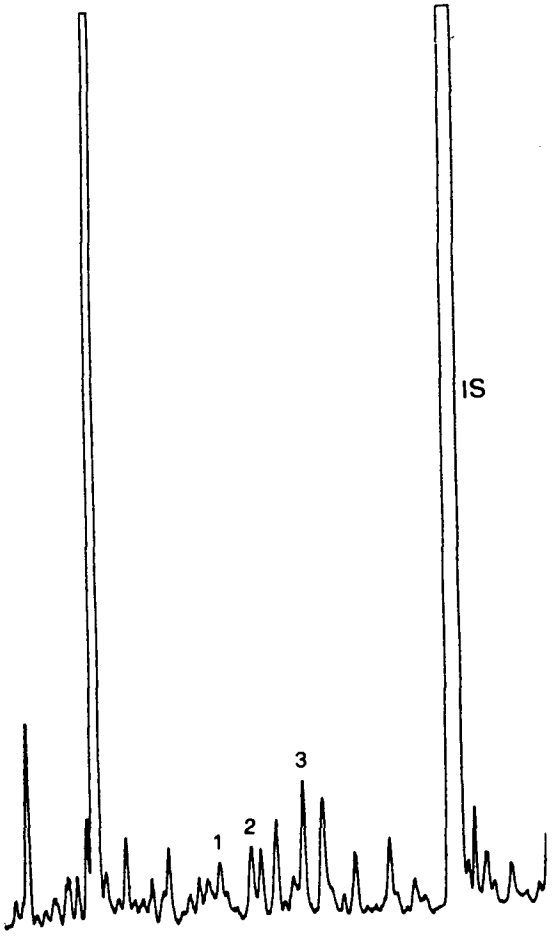

FIG. 3. The dehydroxylated sterol region of the gas chromatogram of a sample of butter spiked with 2% refined soybean oil. 1, 3,5-Campestadiene; 2, 3,5-stigmastatriene; 3, 3,5-stigmastadiene; IS, internal standard.

This study demonstrated the feasibility of using the analysis of hydrocarbons, derived from the dehydroxylation of sterols, for the detection of low levels of refined

fat, such as beef tallow and vegetable oils, in adulterated milkfat.

The 3,5-cholestadiene from cholesterol can be considered an index for the presence of refined beef tallow in butter. Based on our results, the minimum detectable concentration of beef tallow was less than 1%. However, the following factors have to be taken into account—genuine butter can contain small concentrations of 3,5-cholestadiene, and the concentration of this compound in refined beef tallow is closely related to the refining conditions (amount of bleaching earth, temperature, etc.). Therefore, the accuracy of this method has to be confirmed by analyzing a larger number of genuine butter and beef tallow samples.

The dehydroxylation of phytosterols, which are characteristic of vegetable oil, produces several hydrocarbons, which can be used as an index for the addition of refined oil to milkfat. The absence of dehydrophytosterols in butter improves the detection of vegetable oil. It also probably allows vegetable fat that was subjected to desterolyzation to be detected.

The method described in this paper is not affected by the natural variability of milkfat constituents. In fact, it is based on the determination of the compounds produced during refining treatments. Because most adulterating fats have to be refined prior to addition to butter, the evaluation of dehydroxylated sterols can be useful to safeguard a prized product, such as butter.

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