RAPID VOLUMETRIC DETERMINATION OF FAT IN MILK CARAMELS

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By

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The amount of total fat in market milk caramels have been given generally as from 3 to 10 per cent. The fat exists in caramels as colloidal suspensions containing milk and other fats and oils, and the contents will affect the several qualities, of which flavor, texture and taste are probably the most important. On many problems of caramel making, therefore, the rapid estimation of fat in the caramels is desirable.

The most common methods for determining fat content in milk caramels may be classified broadly under three headings: (a) Modified Schmid-Bondzynski process, which relies on acid digestion of the solid-not-fat followed by solvent extraction of fat, (b) Röse-Gottlieb process, which is not submitted to any drastic pretreatment and considered to be the best, (c) Soxlet process, which relies on separation of fat and protein by Rithausen's reagent before the solvent extraction of fat. Recently, the modifications have been adopted for these methods by Voorst(1), Heuser and Krapohl(2), Tevier and Berner(3). Although these methods give fairly correct results, they are still liable to take too much time and too laborious to be practical for routine analysis.

Modifying the saponification method based on another principle which was performed for the determination of fat in faeces by van de Kamer, Huinink and Weyers(4), the fat content in milk caramels could be rapidly determined. Fukuba, Yamazawa and Inagaki (5), Akiya and Nakayama (6) have reported the saponification method for the determination of fat in foods, but they have not tested it for caramels by these methods. In this report, the author has dealt with the availability of the method as described above for the determination of fat in milk caramels.

Method

The procedure is essentially the same as the one described in the report of van de Kamer et al, and the following modified method has been adopted.

2.5 g of caramel is weighed in a 200 ml joint Soxlet flask, and then the caramel is thoroughly dissolved in 5 ml of hot water. After adding 5 ml of 50 per cent potassium hydroxide and 40 ml of ethanol containing 0.4 per cent isoamylalcohol, the mixture is boiled for 30 minutes in boiling water bath under a reflux glass tube (150 × 0.7 cm). Then the flask is cooled, and 17 ml of 25 per cent hydrochrolic acid is added. After the mixture is thoroughly cooled, exactly 50 ml of petroleum ether (b. p. 40~60°) is added and the flask is shaken vigorously for about 1 minute in order to extract completely the fatty acid liberated from the saponified material. 25 ml of the petroleum ether layer is transferred exactly into a small flask from the separated supernatant. After the petroleum ether is evaporated, 10 ml of neutral 98 per cent ethanol is added and the solution is titrated with 0.1 N sodium hydroxide in the presence of 2 per cent alcoholic thymol blue solution as indicator until the color begins to change from yellow to dark blue.

The caluculation is carried out according to the following equation.

$$\text{Fat}\ (\%) = 5.907\ A/Q$$

A ..... the ml of 0.1 N NaOH used in titration
Q ..... the g of caramel taken for analysis

Results

(1) Pretreatment for saponification

It is to be desired that the caramels are thoroughly dissolved in alkaline solution to obtain the correct results. In employing the methods of previous workers, the present author soon encountered a serious difficulty.

When 33 per cent potassium hydroxide is used directly to dissolve the caramel, the it is difficult to dissolve the sample in this solution and takes too much time. In some cases, the mixture changes to gelly-like material is not readily mixed with the ethanol as solvent for the fat.

Since these knotty points of the procedure of previous workers have been resolved by dissolving the caramel in a small amount of water before the potassium hydroxide treatment, the following modification has been adopted. 2.5 g of sample is allowed to dissolve thoroughly in 5 ml of water by stirring the boiling water bath for some time. After cooling it, 5 ml of 50 per cent potassium hydroxide and 40 ml of ethanol containing 0.4 per cent isoamyl-alcohol are added while stirring.

(2) As to the effect of the time required for saponification

Van de Kamer *et al* stated the heating of it for 20 minutes in the course of saponification, and others have shown that 30 minutes' boiling is required for the sufficient saponification. In these cases, they have decided the time

described above for faeces, *miso* (fermented soy-paste), soy bean and rape seed. Table 1 shows the rates of saponification of some caramels with different levels of fat. The saponification for these samples is complete after 30 minutes, no more fat is saponified after heating for 45 or 60 minutes. Therefore, 30 minutes' boiling appears to be also suitable for the saponification of fat in milk caramels.

Table 1. Relation between the rate of saponification and heating time.

Sample number	Fat content (%)			
	Heating time (min)			
	15	30	45	60
1	9.62	10.40	10.45	10.39
2	6.65	7.31	7.27	7.37
3	4.03	4.56	4.60	4.58
4	2.67	3.42	3.39	3.47

(3) Comparison with the other methods

The results of determinations carried out by this method and the other well known methods are shown in table 2. The other methods are as follows:

(1) Soxlet method(7): 3~3.5 g of sample weighed accurately is dissolved in 150 ml of water, mixed with 10 ml of Fehling's copper sulphate solution (34.6 g $CuSO_45H_2O$ dissolve in 500 ml of water) and 7 ml of Rithausen's sodium hydroxide solution (10.2 g of sodium hydroxide dissolve in 1000 ml of water), and filtered with filter paper of Toyo No. 5B. The residue collected in filter paper is dried at 80~100°C, and extracted in Soxlet apparatus with ether for 6 hours.

(2) Röse-Gottlieb method: 2~2.5 g (accurately weighed) of sample is dissolved with 10 ml of water in Roehrig tube, and then subjected to the ordinary treatment.

Besides these methods described above, Babcock method was tested with various treatments, but any satisfactory results had never been got. It appears that the failure is attributed to the drastic action of sulfuric acid.

The results, shown in table 2, indicate that the saponification method has

Table 2. Fat content of milk caramels obtained by some methods.

Sample number	Fat content (%)		
	Soxlet method	R.-G. method	This method
1	9.21	9.24	9.18
2	8.52	8.55	8.50
3	7.64	7.65	7.64
4	4.43	4.47	4.45
5	3.19	3.24	3.17
6	2.56	2.62	2.48

a similarly satisfactory degree of precision compared with the other methods for the determinative indivisuals.

(4) Reliability of the determination

The three samples were subjected to the determination following this saponification method for the decision of reliability of the method. The determination is duplicated ten times per a sample. These statistical data are shown in table 3. The results obtained show that the saponification method is quite available for the determination of fat in milk caramels with an error not exceeding 8.5 per cent.

Table 3. Reliability of this method.

Sample	1	2	3
Mean	11.525	8.642	3.534
Standard deviation	0.24	0.28	0.30
Confidence limit ($\pm$, $t_{0.05}$)	0.170	0.200	0.214
Coefficient of variation (%)	2.08	3.24	8.58

(5) Fat content of some milk caramels (8)

The fat content of some market milk caramels got from market in January in 1956. The results obtained are shown in table 4.

Table 4. Fat content of some market milk caramels.

Sample	Plant	Fat content (%)
1	A	11.51
2	A	11.75
3	B	8.66
4	B	8.52
5	C	3.74
6	C	3.47

Summary

The rapid determination of the fat in milk caramels is performed. The method is based on the principles reported by van de Kamer *et al* for the determination of fat in faeces. In this method, the samples must be dissolved thoroughly in water before the addition of alcoholic potassium hydroxide for saponification and the saponification of fat must be carried out for 30 minutes in boiling water. This method gives practically the same result as the other well known methods and has a satisfactory reliability for the determination of fat in milk caramels.

As the examples, the fat content of some caramels was determined by this method.

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References

1) F. Th. van Voorst (1949). Chem. Weekblad., **45**, 295.
2) G. F. Heuser and E. Krapohl (1941). Z. Untersuch. Lebensm., **82**, 145, Chem. Abst., **38**, 2132.
3) J. Tevier and Ch. Berner (1944). Mitt. Lebensm. Hyg., **35**, 37.
4) J. H. van de Kamer, H. ten B. Huinink and H. A. Weyers (1949). J. Biol. Chem., **177**, 347.
5) H. Fukuba, N. Yamazawa and C. Inagaki (1954). J. Agr. Chem. Soc. Japan, **28**, 59. (in Japanese)
6) T. Akiya and O. Nakayama (1954). Report of the Food Res. Inst., No. 9, 3. (in Japanese)
7) H. Watanabe S. Hase, Y. Hando, S. Matsumoto and H. Aida (1952). Report of the Food Res. Inst., No. 6, 23. (in Japanese)
8) S. Adachi and T. Nakanishi (1957): J. Agr. Chem. Soc. Japan, **31**. 189, (in Japanese)