

MAFF VALIDATED METHODS FOR THE ANALYSIS OF FOODSTUFFS

No. V10

MILK FAT IN COCOA AND CHOCOLATE PRODUCTS

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1. Scope and Field of Application

The method allows the determination of the milk fat content of milk chocolate.

2. Definition

Milk fat: the content of milk fat as determined by the method specified.

3. Principle

The method relies on the fact that milk fat is unique amongst the commonly occurring fats in that it contains *n*-butyric acid.

The fat, extracted from the milk chocolate sample, is saponified. The water-soluble fatty acids are separated by isothermal gas-liquid chromatography in the presence of an internal standard. The *n*-butyric acid is thus determined and used as an index of the milk fat content.

4. Reagents

During the analysis, unless otherwise stated, only use reagents of a recognised analytical grade and distilled water or water of equivalent purity.

4.1 Anti-bumping granules, defatted powdered pumice.

4.2 Ethanolic potassium hydroxide, 0.5 mol/l.

Dissolve potassium hydroxide (**4.2.1**) in ethanol (**4.2.2**).

4.2.1 Potassium hydroxide, pellets.

4.2.2 Ethanol, 96% (V/V).

4.3 *ortho*-Phosphoric acid solution, 5% (m/V).

Dissolve *ortho*-Phosphoric acid (**4.3.1**) in water.

4.3.1 *ortho*-Phosphoric acid, 85% (m/m)

4.4 *n*-Valeric acid, stock solution

Weigh accurately between 240 and 260 mg of *n*-valeric acid (**4.4.1**) and dilute to 100 ml with water.

4.4.1 *n*-Valeric acid, chromatographically pure.

4.5 *n*-Butyric acid, stock solution

Weigh accurately between 380 and 420 mg of *n*-butyric acid (**4.5.1**) and dilute to 100 ml with water.

4.5.1 *n*-Butyric acid, chromatographically pure and preferably assayed by titration.

4.6 Internal standard solution

Make an accurate 10 fold dilution of an aliquot of the *n*-valeric acid stock solution (4.4), to give an aqueous standard solution containing approximately 0.25 mg/ml. 200 ml of this solution should be adequate.

4.7 *n*-Butyric acid standard solution

Make an accurate 10 fold dilution of an aliquot of the *n*-butyric acid stock solution (4.5), to give an aqueous standard solution containing approximately 0.4 mg/ml. 100 ml of this solution should be adequate.

4.8 Mixed standard solutions

Prepare a series of seven standard mixtures of *n*-valeric and *n*-butyric acids by mixing 2.0 ml of internal standard solution (4.6) with 5.0, 3.5, 2.0, 1.00, 0.50, 0.20 and 0.00 ml of *n*-butyric acid standard solution (4.7), and making each up to 7.0 ml by adding the calculated volume of water. Calculate the actual concentrations of *n*-valeric and *n*-butyric acids from the accurate weight of the acids taken in the preparation of the stock solutions (4.4, 4.5); the nominal weights per 7 ml of each mixed standard solution are 0.50 mg *n*-valeric acid and respectively 2.00, 1.40, 0.80, 0.40, 0.20, 0.08, and 0.00 mg *n*-butyric acid. These solutions should be stored in a refrigerator and will be stable for up to a month.

5. Apparatus

5.1 Water-bath, electrically heated or steam-bath.

5.2 Beakers, 50 ml, with watch glasses to cover.

5.3 Analytical balance, sensitive to 0.1 mg

5.4 Test-tubes, 10 ml, with ground-glass stoppers.

5.5 Graduated pipettes, 5 ml and 1 ml.

5.6 Volumetric pipettes, 5 ml and 2 ml.

5.7 Fluted filter papers, diameter 90 mm, slow.

5.8 Filter funnels, to accept filter paper (5.10).

5.9 Oven, at 100-101°C

5.10 Gas chromatograph, able to accept dual 1.5 m x 6 mm (o.d.) (5 ft x 1/4") glass columns with on-column injection, and fitted with flame ionisation detectors and a potentiometric recorder with chart width preferably not less than 200 mm.

5.10.1 Specified chromatographic conditions

1.5 m x 6 mm (o.d.) (5 ft x 1/4") glass column packed with 5% Carbowax 20M and 0.5% terephthalic acid on 100-120 mesh, acid-washed Supasorb; nitrogen flow rate, 50 ml/min; column temperature 125°C (isothermal); flame ionisation detectors: hydrogen flow rate, 50 ml/min; air flow rate, 500 ml/min.

5.10.2 Preparation of recommended column packing

Reflux 100 ml of ethanol with 4 g of Carbowax 20M and 0.4 g of terephthalic acid until dissolved. Add 20 g of Supersorb (eg BDH) and boil under reflux to remove air. Filter rapidly at the pump (approximately 25 ml of the solution is retained by the Supersorb) and dry the residue under vacuum. After packing the columns, purge with nitrogen at 220°C for 24 hr.

6. Procedure

6.1 Extraction of fat

Extract the fat using an appropriate procedure.

6.2 Saponification

Ensure that the fat is homogeneous by melting and mixing it before taking a sample aliquot. Weigh 100-110 mg of this fat sample accurately (W mg) into a 50 ml beaker. Add 3 ml of ethanolic potassium hydroxide (4.2), cover the beaker with a watch glass and immediately place on a boiling water-bath or steam-bath. Heat for at least 10 min, or until no fat globules are visible on the surface of the liquid. Remove the watch glass and continue heating until the ethanol has completely evaporated. Allow the beaker to cool and add 5.0 ml of water, cover with a watch glass and shake carefully to dissolve the soap completely. It may occasionally be necessary to warm the mixture gently to encourage the dissolution of the soaps.

6.3 Preparation of acids

Add 5.0 ml of *ortho*-phosphoric acid solution (4.3) to the beaker and swirl gently to coagulate the precipitated higher fatty acids. Filter through a fluted filter paper. Pipette 5.0 ml of filtrate and 2.0 ml of internal standard solution into a test tube and mix.

6.4 Injection of acids

Inject a nominal 1 µl of the final solution onto the GLC column, using an on-column injection technique. Measure the peak heights corresponding to *n*-butyric and *n*-valeric acids to the nearest 0.5 mm. The micro-syringe should be flushed occasionally with distilled water to prevent corrosion of the plunger by residual phosphoric acid.

6.5 Construction of the calibration curve

Inject a nominal 1 µl of each of the seven mixed standard solutions (4.8) and measure peak heights as in 6.4. Plot a calibration curve relating the weight of *n*-butyric acid (mg) in 7 ml of mixed standard solution (4.8) to the corresponding peak height ratio, PHR, defined in Section 8.

7. COSHH

Analysts are reminded that appropriate hazard and risk assessments required by the Control of Substances Hazardous to Health Regulations, 1988 (See "Control of Substances Hazardous to Health - Approved Code of Practice, Control of Substances Hazardous to Health Regulations, 1988") must be made before using this method.

8. Expression of Results

For each chromatogram, calculate the peak height ratio, PHR, defined by:

$$\text{PHR} = \frac{\text{Peak Height corresponding to } n\text{-butyric acid (mm)}}{\text{Peak Height corresponding to } n\text{-valeric acid (mm)}}$$

From the calibration curve (6.5), read off the weight of *n*-butyric acid, *M* mg, corresponding to the peak height ratio calculated from the sample chromatogram (6.4).

The original sample of fat (of weight *W* mg) therefore contains $2 \times M$ mg of *n*-butyric acid; the level of *n*-butyric acid in the extracted fat is:

$$\% \text{ } n\text{-butyric acid content of fat} = 100 \times (2 \times M/W)$$

If a concentration of 3.60 mg of *n*-butyric acid in 100 mg of any milk fat is taken as an arbitrary average value when determined by this method, then the concentration of milk fat in the extracted fat is given by:

$$\% \text{ milk fat content of fat} = 100 \times 100 \times 2 \times M / (W \times 3.60)$$

The milk fat content of the original sample of milk chocolate is then given by:

$$\% \text{ milk fat content of chocolate} = A \times 100 \times 2 \times M / (W \times 3.60)$$

where:

A is the % total fat content of original sample of cocoa or chocolate product. (This value can be determined using Method No. V9 "Total fat in cocoa and chocolate products" of the MAFF Validated Methods for the Analysis of Foodstuffs series.)

9. References

- 9.1 HJ Judd, MD Percival and R Wood, J. Assoc. Pub. Analysts, 1984, **22**, 81-101.
- 9.2 DO Biltcliffe and R Wood, J. Assoc. Pub. Analysts, 1982, 20, 69-88.
- 9.3 AR Phillips and BJ Sanders, J. Assoc. Pub. Analysts, 1968, 6, 89-95.
- 9.4 Ministry of Agriculture, Fisheries and Food, Food Safety Directorate, MAFF Validated Methods for the Analysis of Food, Introduction, General Considerations and Analytical Quality Control, J. Assoc. Publ. Analysts, 1992, **28**, 11-16

APPENDIX 1

Analytical Quality Control

General principles of analytical quality control are outlined protocol V.0 of the series⁽⁴⁾.

A1 Repeatability

The absolute difference between two test results obtained under repeatability conditions should not be greater than the repeatability, *r*, deduced from the collaborative trial data summarised below (Table 1). At levels of milk fat of 6-7%, *r* may be taken as 0.6%. At lower levels the repeatability deteriorates. The overall relative standard deviation of

repeatability (coefficient of variance of repeatability), RSD_r , for levels of milk fat of 6-7%, may be taken as less than 3%.

A2 Reproducibility

The absolute difference between two test results obtained under reproducibility conditions should not be greater than the reproducibility, R , deduced from the collaborative trial data summarised below (Table 1). The observed reproducibility was not as satisfactory as expected; R may be taken as 2.0%. This precision corresponds to an overall relative standard deviation of reproducibility (coefficient of variance of reproducibility), RSD_R , of up to 13%.

A3 Trueness (Bias)

The collaborative trial established precision parameters for the method. Its accuracy depends crucially on the factor chosen to convert *n*-butyric acid into milk fat levels; considerable bias can be introduced by the use of an inappropriate conversion factor. The arbitrary choice of 3.60 arises from the milk fat used for a preliminary study (8.2), and does not necessarily represent the figure of choice for a statutory procedure. Nevertheless results based on it will be useful if treated with caution. A previous study of this method (8.3) reported the observed levels of *n*-butyric acid in 19 different samples of butter fat; the average level was 3.62% *m/m*, but the extremes were 3.38-4.00% (*m/m*). The use of this average value as a basis for milk fat estimation appears unlikely to introduce an error of much more than 10% of the observed value.

Comparison in Table 1 between the observed mean and the approximate recipe values of total fat content suggests satisfactory accuracy at 6-7% milk fat levels. Using an arbitrary factor of 3.60, recoveries with samples B/E and C/F were 92-94%. At lower levels, the observed recovery was unexpectedly high (145%), suggesting that this factor did not correspond to the milk ingredient of sample A/D.

A4 Limit of Detection

This limit has not been established, but the collaborative trial data suggest an accuracy which, if maintained, corresponds to an extrapolated lower limit of roughly 2% milk fat for a single determination.

A5 Statistical Data Derived from the Results of Interlaboratory Tests

Participants in the collaborative trial each analysed six subsamples of milk chocolate once (three different samples in blind duplicate). The approximate nominal milk fat content was known from the recipe of each sample.

The stipulated conditions of gas-liquid chromatography (5.12.1) were recommended and specified by MAFF (8.1) to the participants in the collaborative trial. Other similar conditions may be suitable (8.2, 8.3).

Table 1 summarises the statistical data; the milk fat contents are expressed as a percentage by mass of the sample.

TABLE 1
Statistical Analysis of the % Milk Fat in Milk Chocolate Samples

Sample	A/D	B/E	C/F
Number of Laboratories retained after eliminating outliers	16	12	14
Number of Laboratories eliminated as outliers	2	6	4
LEVEL OF ANALYTE			
Nominal Recipe Value	3.7	7.5	6.7
Mean observed value $\bar{x}$	5.36	6.91	6.31
REPEATABILITY			
Standard Deviation S_r	0.56	0.20	0.14
Relative Standard Deviation RSD_r (%)	10.5	2.9	2.3
Repeatability r [$2.8 \times S_r$]	1.57	0.56	0.40
REPRODUCIBILITY			
Standard Deviation S_R	0.69	0.40	0.66
Relative Standard Deviation RSD_R (%)	12.9	5.7	10.5
Reproducibility R [$2.8 \times S_R$]	1.93	1.11	1.85

A6 Key to Table 1

Symbol	Definition
$\bar{x}$	Overall mean value
S_r	The standard deviation of repeatability
RSD_r	The relative standard deviation of repeatability, expressed as a percentage of the mean (coefficient of variance of repeatability CV_r)
r	Repeatability
S_R	The standard deviation of reproducibility
RSD_R	The relative standard deviation of reproducibility, expressed as a percentage of the mean (coefficient of variance of reproducibility CV_R)
R	Reproducibility