

MAFF VALIDATED METHODS FOR THE ANALYSIS OF FOODSTUFFS

No. V7

UNSAAPONIFIABLE MATTER IN COCOA AND CHOCOLATE PRODUCTS

Correspondance on this method may be sent to R. Wood, Statutory Methods (Chemistry and Microbiology) Department, Ministry of Agriculture, Fisheries and Food, Food Science Laboratory, Food Safety Directorate, Norwich Research Park, Colney, Norwich NR4 7UQ

1. Scope and Field of Application

The method allows the determination of the unsaponifiable matter of cocoa butter.

2. Definition

Content of unsaponifiable matter: the content of unsaponifiable matter as determined by the method specified.

3. Principle

The sample is saponified and then extracted with petroleum ether. The extract is dried and then weighed.

4. Reagents

Wherever the use of water is required, distilled or water of equivalent purity is to be used.

4.1 Ethanol, 95% (V/V).

4.2 Potassium hydroxide solution: dissolve 60 g of potassium hydroxide in 40 ml water.

4.3 Ethanol, dilute: 50% (V/V).

4.4 Petroleum ether: petroleum ether, boiling range 40-60°C, redistilled.

4.5 Phenolphthalein solution, 1% (m/V) in ethanol (4.1).

4.6 Acetone

5. Apparatus

5.1 Oven, electrically heated, thermostatically controlled at $52.5 \pm 2.5^\circ\text{C}$.

5.2 Water bath, boiling.

5.3 Separators, 250 ml, each fitted with a teflon stopcock and stopper.

5.4 Desiccator, containing freshly activated silica gel (with water content indicator), or an equivalent desiccant.

5.5 Flasks, 250 ml with ground glass joints.

5.6 Flasks, 50 ml.

5.7 Reflux condenser, with ground glass joints.

5.8 Oven, electrically heated, thermostatically controlled at $102.5 \pm 2.5^\circ\text{C}$.

5.9 Analytical balance

All ground glass joints should be free from lubricants; they are to be cleansed with petroleum ether before starting the analysis. Do not handle the flasks by hand but use tongs, etc.

6. Procedure

6.1 Fat saponification

Melt the fat sample in the oven at $52.5 \pm 2.5^\circ\text{C}$ and then filter. Accurately weigh to the nearest 0.1 mg about 5 g of the filtered fat into a 250 ml ground glass stoppered flask. Add 50 ml of ethanol and 10 ml of potassium hydroxide solution. Attach a reflux condenser to the flask, place on a boiling water bath and heat for 1 hr. Stop the heating. Add 50 ml water through the top of the condenser, shake and cool to room temperature. Check that the solution does not contain unsaponified fat droplets.

6.2 Extraction

Transfer the solution obtained to a 250 ml separator. Rinse the condenser and flask with five 10 ml portions of petroleum ether and add to separator. Shake vigorously for 1 min., periodically releasing the pressure by inverting the separator and opening the stopcock. Allow to stand until phase separation is nearly complete (usually about 10 min.). Drain the soap solution as completely as possible into a second 250 ml separator ensuring that no petroleum ether is transferred. If an emulsion forms, break it by the addition of a few millilitres of ethanol.

Repeat the extraction of the soap solution twice with two 50 ml portions of petroleum ether; combine all the petroleum ether extracts and wash three times with 50 ml portions of dilute ethanol (50% V/V).

Drain each wash solution to about 2 ml, ensuring that no petroleum ether is transferred. Then gently rotate the separator and let the layers separate for 5 - 10 min. Drain the remaining wash solution, closing the stopcock when the petroleum ether starts to pass the bore of the stopcock. Check the last wash with phenolphthalein: if alkali is present, wash again and recheck.

6.3 Weighing of unsaponifiable matter

Transfer the petroleum ether solution into a 250 ml flask through the top of the separator. Rinse the separator and its pouring edge twice with 10 ml of petroleum ether and add the rinsings to the main solution. Evaporate to approximately 5 ml on the water bath. Transfer quantitatively, rinsing three times with 3-5 ml portions of petroleum ether, to a 50 ml flask which has been previously dried by heating for not less than 1 hr. in the oven at $102.5 \pm 2.5^\circ\text{C}$, cooled in a desiccator for not less than 1 hr. and then weighed to 0.1 mg.

Place the flask with its contents on the water bath and evaporate to dryness. Add 2 - 3 ml acetone and remove all solvent on the boiling water bath with a gentle stream of air or nitrogen passing through the flask. Place the flask horizontally in the oven at $102.5 \pm 2.5^\circ\text{C}$ and dry for 30 min. Remove and cool in a desiccator for not less than 1 hr.; then weigh.

Repeat the drying, cooling and weighing process until the change in weight is not more than 1.5 mg. Discard the sample if the weight after the third weighing varies by more than 1.5 mg from the second.

6.4 Blank

Repeat the whole procedure but omitting the fat sample to obtain the blank weight

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

The percentage of unsaponifiable matter content is given by:

$$\% \text{ unsaponifiable matter content} = 100 \times (m_1 - m_2) / m_0$$

Where:

- m_1 is the mass in grams of the residue;
- m_2 is the mass in grams of the blank;
- m_0 is the mass in grams of the sample.

9. References

- 9.1 HJ Judd, MD Percival and R Wood, J. Assoc. Publ. Analysts, 1984, **22**, 81-101.
- 9.2 "Determination of unsaponifiable matter in cocoa butter", International Office of Cocoa and Chocolate/International Sugar Confectionery Manufacturers' Association (IOCC/ISCMA) Analytical Method 102B, 1977. London.
- 9.3 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 in protocol V0 of the series⁽³⁾.

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). For unsaponifiable contents within the range 0.27% - 0.55%, r may be taken as 0.10 - 0.15%. This precision corresponds to a relative standard

deviation of repeatability (coefficient of variance of repeatability), RSD_r , of 10 - 14%.

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). For unsaponifiable contents within the range 0.27 - 0.55%, R may be taken as 0.09 - 0.21%. This precision corresponds to a relative standard deviation of reproducibility (coefficient of variance of reproducibility), RSD_R , of 12 - 14%.

A3 Trueness (Bias)

The collaborative trial established satisfactory precision parameters for the method, but its accuracy was not tested by spiking with known amounts of authentic unsaponifiable matter. However, there is no reason to suspect systematic bias.

A4 Limit of Detection

This limit has not been established, but the collaborative trial data suggests an accuracy which, if maintained, corresponds to an extrapolated lower limit of roughly 0.1% unsaponifiable matter for a single determination.

A5 Statistical Data Derived from the Results of Interlaboratory Tests

Participants in the collaborative trial each analysed six samples of cocoa butter once (three samples in blind duplicate). One pair of samples (V/X) had been pressed from roasted nib; another (T/Z) was V/X spiked with 1% m/m stearic acid; the third (W/Y) pair was solvent-extracted cocoa butter, expected to contain higher amounts of unsaponifiable matter.

Table 1 summarises the statistical data; the unsaponifiable matter content was expressed as a percentage by mass of the sample.

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

TABLE 1
Statistical Analysis of the % of Unsaponifiable Matter in Cocoa Butter Samples

Sample	T/Z	V/X	W/Y
Number of Laboratories retained after eliminating outliers	16	16	18
Number of Laboratories eliminated as outliers	2	2	0
Number of results accepted after eliminating outliers	32	32	36
LEVEL OF ANALYTE			
Mean observed value $\bar{x}$	0.28	0.27	0.55
REPEATABILITY			
Standard Deviation S_r	0.39	0.32	0.57
Relative Standard Deviation RSD_r (%)	14	12	10
Repeatability r [$2.8 \times S_r$]	0.11	0.09	0.16
REPRODUCIBILITY			
Standard Deviation S_R	0.039	0.032	0.075
Relative Standard Deviation RSD_R (%)	14	12	14
Reproducibility R [$2.8 \times S_R$]	0.11	0.09	0.21

