

# MAFF VALIDATED METHODS FOR THE ANALYSIS OF FOODSTUFFS

No V9

## TOTAL FAT IN COCOA AND CHOCOLATE PRODUCTS

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### 1. Scope and Field of Application

The method allows the determination of the total fat content of milk chocolate, cocoa mass and cocoa powder.

### 2. Definition

**Total fat:** the total content of fat as determined by the method specified.

### 3. Principle

The product is hydrolysed with diluted hydrochloric acid and filtered. The dried mass containing the fat is extracted with petroleum ether, the solvent evaporated and the residue weighed.

### 4. Reagents

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

**4.1** Hydrochloric acid, 25% (*m/m*) ( $\rho_{20} = 1.12$ ). Mix two volumes of concentrated hydrochloric acid ( $\rho_{20} = 1.118$ ) with one volume of water.

**4.2** Petroleum ether, boiling range within the range 30-60°C. Dry and redistil if necessary.

**4.3** Silver nitrate solution, 0.1 mol/l.

**4.4** Anti-bumping granules, e.g. powdered pumice which has been previously defatted.

### 5. Apparatus

**5.1** Soxhlet extractor, with siphon capacity about 100 ml, fitted with ground glass joints and a flat-bottomed 250 ml flask.

**5.2** Condenser

**5.3** Filter paper, fluted and of pore size to allow the filtration of the digest at a reasonable speed.

**5.4** Glass wool

**5.5** Extraction thimble, double thickness, fat-free, to fit the Soxhlet extractor.

**5.6** Analytical balance

5.7 Water-bath, electrically heated.

5.8 Oven, at 100-101°C.

## 6. Procedure

### 6.1 Digestion of the sample

Grind material such as cocoa nibs, cocoa shell, press cake, expeller cake, etc, to a maximum of 150 µm (check using a wire mesh), care being taken to avoid liberation of fat during the grinding operation. Weigh the ground sample to the nearest 1 mg into a 300-500 ml beaker: take sufficient sample to yield about 1 g of fat on extraction. Add, while stirring continuously, 45 ml of boiling water followed by 55 ml hydrochloric acid. Add anti-bumping agent. Cover the beaker with a watch glass and boil gently for exactly 15 min., keeping the volume approximately constant by adding water if necessary.

Rinse the watch glass into the beaker with 100 ml of water. Filter the digest through a wet fat-free fluted filter paper, using a Celite filter aid if necessary.

Rinse the beaker three times with hot water, passing the washings through the filter; continue to wash until the filtrate ceases to react on addition of silver nitrate solution. Transfer the wet filter paper and residue to a fat-free extraction thimble. Place a glass wool plug over the filter paper and dry for 6-18 hr. in a small beaker in an oven at 100-101°C. Also dry the first beaker and the watch glass.

### 6.2 Extraction of fat

Dry the flat-bottomed 250 ml flask containing a few anti-bumping granules in an oven at 100-101°C for 1 hr. Cool, weigh to the nearest 0.1 mg and connect to the Soxhlet extractor. Support the thimble on a spiral or on glass balls to ensure efficient working of the siphon. Wash both beakers and the watch glass with about 150 ml petrol, and pour the solvent gradually into the thimble. Extract under reflux until extraction is complete: this may be verified by replacing the weighed flask with a second dry weighed flask and repeating the extraction of fat operation, extracting for about 2 hr. There should be less than 1 mg of fat recovered in the second flask.

Evaporate the solvent over a boiling water-bath and dry the flask containing the fat in an oven at 100-101°C. Weigh and repeat the drying operation until the variation in weight of two consecutive weighings is less than 1 mg.

## 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 total fat content expressed as a percentage by mass of the prepared sample is given by:

$$\% \text{ total fat} = 100 \times (m_2 - m_1)/m_0$$

where

- $m_0$  is the mass in g of the sample;
- $m_1$  is the mass in g of the dried flask;
- $m_2$  is the mass in g of the flask and the extracted fat.

## 9. References

- 9.1 HJ Judd, MD Percival and R Wood, J. Assoc. Publ. Analysts, 1984, **22**, 81-101.
- 9.2 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<sup>(2)</sup>.

#### 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 (Tables 1-3). For total fat contents within the range 10%-60%,  $r$  may be taken as 0.4-0.9%, though the observed value of  $r$  was somewhat greater (1.5) with one of the cocoa mass samples. The relative standard deviation of repeatability (coefficient of variance of repeatability),  $RSD_r$ , was less than 1% when analysing chocolate or cocoa mass samples, but 1-3% with cocoa powder.

#### 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 (Tables 1-3). For total fat contents within the range 10-60%,  $R$  may be taken as 0.8-1.9%. This precision corresponds to a relative standard deviation of reproducibility (coefficient of variance of reproducibility),  $RSD_R$ , of about 1% (2-5% with cocoa powder).

**A3 Trueness (Bias)**

The collaborative trial established satisfactory precision parameters for the method. Comparison in Tables 1-3 between the observed mean and the approximate recipe values of total fat content suggests satisfactory accuracy. While observed values tended to be low, they rarely differed by more than 2% fat from the expected value, which was itself subject to uncertainty.

**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 1% 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). They similarly analysed three different samples of cocoa mass and three of cocoa powder, each in blind duplicate. The approximate nominal total fat content was known from the recipe of each sample.

Tables 1, 2 and 3 summarise the statistical data for the milk chocolate, cocoa mass and cocoa powder respectively; the total fat content is expressed as a percentage by mass of the sample.

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

| Sample                                                     | A/D   | B/E   | C/F   |
|------------------------------------------------------------|-------|-------|-------|
| Number of Laboratories retained after eliminating outliers | 16    | 17    | 16    |
| Number of Laboratories eliminated as outliers              | 2     | 1     | 2     |
| Number of results accepted after eliminating outliers      | 32    | 34    | 32    |
| LEVEL OF ANALYTE                                           |       |       |       |
| Nominal Recipe Value                                       | 27.5  | 30.0  | 37.5  |
| Mean observed value $\bar{x}$                              | 29.29 | 29.43 | 35.64 |
| REPEATABILITY                                              |       |       |       |
| Standard Deviation $S_r$                                   | 0.17  | 0.15  | 0.15  |
| Relative Standard Deviation $RSD_r$ (%)                    | 0.58  | 0.51  | 0.42  |
| Repeatability $r$ [ $2.8 \times S_r$ ]                     | 0.47  | 0.43  | 0.42  |
| REPRODUCIBILITY                                            |       |       |       |
| Standard Deviation $S_R$                                   | 0.31  | 0.29  | 0.36  |
| Relative Standard Deviation $RSD_R$ (%)                    | 1.06  | 0.99  | 1.01  |
| Repeatability $R$ [ $2.8 \times S_R$ ]                     | 0.87  | 0.82  | 1.02  |

**TABLE 2**  
**Statistical Analysis of the % Fat in Cocoa Mass Samples**

| Sample                                                     | N/S   | O/R   | P/Q   |
|------------------------------------------------------------|-------|-------|-------|
| Number of Laboratories retained after eliminating outliers | 16    | 15    | 17    |
| Number of Laboratories eliminated as outliers              | 2     | 3     | 1     |
| Number of results accepted after eliminating outliers      | 32    | 30    | 34    |
| LEVEL OF ANALYTE                                           |       |       |       |
| Nominal Recipe Value                                       | 56    | 56    | 57    |
| Mean observed value $\bar{x}$                              | 55.17 | 54.25 | 54.88 |
| REPEATABILITY                                              |       |       |       |
| Standard Deviation $S_r$                                   | 0.32  | 0.26  | 0.52  |
| Relative Standard Deviation $RSD_r$ (%)                    | 0.58  | 0.48  | 0.95  |
| Repeatability $r$ [ $2.8 \times S_r$ ]                     | 0.91  | 0.73  | 1.47  |
| REPRODUCIBILITY                                            |       |       |       |
| Standard Deviation $S_R$                                   | 0.59  | 0.39  | 0.67  |
| Relative Standard Deviation $RSD_R$ (%)                    | 1.07  | 0.72  | 1.22  |
| Repeatability $R$ [ $2.8 \times S_R$ ]                     | 1.66  | 1.10  | 1.88  |

**TABLE 3**  
**Statistical Analysis of the % Fat in Cocoa Powder Samples**

| Sample                                                     | G/M   | H/L   | J/K   |
|------------------------------------------------------------|-------|-------|-------|
| Number of Laboratories retained after eliminating outliers | 15    | 15    | 18    |
| Number of Laboratories eliminated as outliers              | 3     | 3     | 0     |
| Number of results accepted after eliminating outliers      | 30    | 30    | 36    |
| LEVEL OF ANALYTE                                           |       |       |       |
| Nominal Recipe Value                                       | 16    | 21    | 11    |
| Mean observed value $\bar{x}$                              | 14.91 | 19.11 | 10.37 |
| REPEATABILITY                                              |       |       |       |
| Standard Deviation $S_r$                                   | 0.20  | 0.22  | 0.32  |
| Relative Standard Deviation $RSD_r$ (%)                    | 1.43  | 1.15  | 3.09  |
| Repeatability $r$ [ $2.8 \times S_r$ ]                     | 0.55  | 0.61  | 0.90  |
| REPRODUCIBILITY                                            |       |       |       |
| Standard Deviation $S_R$                                   | 0.42  | 0.40  | 0.55  |
| Relative Standard Deviation $RSD_R$ (%)                    | 2.82  | 2.09  | 5.30  |
| Repeatability $R$ [ $2.8 \times S_R$ ]                     | 1.18  | 1.12  | 1.53  |

**A6 Key to Tables 1, 2 and 3**

| 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                                                                                                                                |