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MAFF VALIDATED METHODS FOR THE ANALYSIS OF FOODSTUFFS

No. V16

TOTAL FAT IN MAYONNAISE

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1. Scope and Field of Application

The method allows the determination of the total fat content of mayonnaise and other emulsified sauces.

2. Definition

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

3. Principle

The well-mixed sample is digested with hydrochloric acid and the resulting liquid filtered through two moistened pleated filter papers. The residue remaining on the filter papers is dried and extracted for 4 hr. with petroleum ether or *n*-hexane. The solvent is distilled off and the residual fat is dried at 103 ± 2°C under atmospheric pressure, cooled and weighed. The fat content is calculated from the weight obtained.

4. Reagents

All reagents should be of recognised analytical grade unless specified otherwise.

4.1 Indicator paper

4.2 Petroleum ether, boiling range 40 - 60°C, or *n*-hexane.

4.3 Hydrochloric acid, approximately 4 mol/l.

4.4 Silver nitrate solution, 0.1 mol/l.

4.5 Water, distilled or demineralised.

4.6 Cotton wool, defatted.

5. Apparatus

5.1 Ceramic wire gauze, for Bunsen burner and tripod.

5.2 Beakers, 600 ml, tall form.

5.3 Desiccator, containing silica gel or other suitable drying agent.

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

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5.5 Extraction thimbles, defatted (e.g. Schleicher & Schull No 603 or Macherey & Nagel No 645F) to fit the Soxhlet extractor.

5.6 Double pleated filter papers, 150 - 200 mm diameter with average pore diameter 5 µm maximum (e.g. Schleicher & Schull No 597 1/2 or No 595 1/2, or Macherey & Nagel No 616 1/4 or No 615 1/4).

5.7 Glass rod

5.8 Glass funnel, 100 mm diameter minimum.

5.9 Sand or water bath, with suitable means of controlling heating.

5.10 Anti-bumping granules

5.11 Watch-glass cover, 100 mm diameter.

5.12 Drying oven, electrically heated and thermostatically controlled at 103 ± 2°C.

6. Procedure

6.1 Sample Preparation and Storage

Take the contents of an entire package or several packages to provide a subsample of at least 200 g. Store in a tightly closed container at 2 - 6°C in the dark to prevent any alteration. Allow the sample to reach uniform room temperature before analysis, stirring if necessary.

6.2 Procedure for fat determination

6.2.1 Dry a flat-bottomed extraction flask, containing an anti-bumping granule, in the oven for 1hr. at 103 ± 2°C, cool in a desiccator to room temperature, and weigh; designate as weight *A*.

6.2.2 Weigh (to the nearest mg) 3 - 5 g of the well-mixed sample (depending upon the weight of fat expected, which should not exceed 3 g) into a 600 ml beaker (**5.2**); designate weight of sample as *C*.

6.2.3 Add 150 ml of hydrochloric acid (**4.3**) to the beaker and stir with the glass rod. Add a few anti-bumping granules, cover the beaker with a watch-glass, and heat to boiling. Keep the contents boiling gently on a low heat for 1 hr, stirring frequently.

6.2.4 Add 150 ml of hot water to the beaker. Place the fluted filter papers in the funnel and moisten thoroughly with hot water. Filter the hot digested liquid quickly, and wash the beaker, watch-glass cover and glass rod three times with hot water, passing each successive washing through the filter papers. Use a Celite filter aid if necessary.

Test the washings for absence of acidity, using indicator paper (**4.1**), or for the absence of chloride, using silver nitrate solution (**4.4**). Continue washing the filters until the filtrate is free of acid.

6.2.5 Place the funnel containing the filter papers in the beaker with the watch-glass and glass rod, and dry in the oven for 1 hr.

6.2.6 Transfer the dry filter papers to an extraction thimble. Remove any traces of fat present in the beaker with a piece of cotton wool damped with extraction solvent (**4.2**), and add this to the extraction thimble. Place the thimble in the extraction apparatus, add solvent to the extraction flask, and assemble the extractor. Rinse the beaker, watch-glass cover and glass rod with solvent and add the rinsings to the extraction apparatus. Heat the extraction flask on a sand or water bath, and allow the extraction to proceed continuously for 4 hr.

6.2.7 Remove the bulk of the solvent by distillation, and any traces of solvent remaining with a gentle stream of air. Dry the flask in a horizontal position in the oven for 1 hr. at 103 ± 2°C, cool in the desiccator and weigh to the nearest mg. Repeat the drying, cooling and weighing process until successive weights differ by no more than 1 mg; designate this weight as *B*.

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 sample (i.e. in g/100 g), is given by:

$$\% \text{ total fat content} = 100 \times (B - A) / C$$

where

A is the weight in g of the empty flask and granule (**6.2.1**);

B is the weight in g of the flask with extracted fat after drying (**6.2.7**);

C is the weight in g of sample taken (**6.2.2**).

9. References

9.1 MJ Scotter, V Staniforth and R Wood, J. Assoc. Publ. Analysts, 1989,**26**, 103-115.

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 in protocol V.0 of the series[2].

A1 Repeatability

The absolute difference between two test results obtained under repeatability conditions should not be greater than the repeatability limit, r, deduced from the collaborative trial data summarised below (Table 1). When analysing mayonnaise containing about 75% total fat, the value of r may be taken as 1.1%. This precision corresponds to a relative standard deviation of repeatability (coefficient of variance of repeatability), RSD_r, of about 0.5%.

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 total fat contents of about 75%, R may be taken as 2.0%. This precision corresponds to a relative standard deviation of reproducibility (coefficient of variance of reproducibility), RSD_R, of less than 1%.

A3 Trueness (Bias)

The collaborative trial established satisfactory precision parameters for the method. Comparison in Table 1 between the observed means and the calculated recipe values of total fat content suggests satisfactory accuracy. The mean observed values were always somewhat higher than the "expected" values, but any possible systematic bias may be neglected; the differences were never more than 1.1 g/100 g. Such slight bias could be caused by the occlusion of traces of solvent in the fat residues after drying.

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 theoretical lower limit of roughly 1.1 g/100 g for a single determination.

A5 Statistical Data Derived from the Results of Interlaboratory Tests

Participants in the collaborative trial each analysed six subsamples of mayonnaise once (three different samples in blind duplicate). The total fat content of each sample was also calculated from its recipe.

Table 1 summarises the statistical data; the total fat content is expressed as a percentage by mass of the sample.

TABLE 1

Statistical Analysis of the % Total Fat in Mayonnaise Samples

Sample	1/3	4/6	2/5
Number of laboratories retained after eliminating outliers	16	19	20
Number of laboratories eliminated as outliers	4	1	0
Number of results accepted after eliminating outliers	32	38	40
LEVEL OF ANALYTE			
"Expected" recipe value	75.5	76.6	78.7
Mean observed value	76.6	77.4	79.3
REPEATABILITY			
Standard Deviation S_r	0.37	0.40	0.57
Relative Standard Deviation RSD_r (%)	0.48	0.52	0.72
Repeatability r [2.8 x S_r]	1.03	1.12	1.60
REPRODUCIBILITY			
Standard Deviation S_R	0.68	0.72	1.07
Relative Standard Deviation RSD_R(%)	0.89	0.93	1.35
Reproducibility r [2.8 x S_R]	1.89	2.03	2.99

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