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MAFF VALIDATED METHODS FOR THE ANALYSIS OF FOODSTUFFS

No. V 17

EGG-YOLK IN MAYONNAISE

Correspondence 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 egg-yolk content of mayonnaise and other emulsified sauces.

2. Definition

Egg-yolk content: the content of egg-yolk as determined by the method specified.

3. Principle

The phospholipids are extracted together with fat using a mixture of chloroform and ethanol. After ashing, the phosphate content is determined gravimetrically as the quinoline phosphomolybdate.

4. Reagents

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

4.1 Ethanol, 96% (*V/V*).

4.2 Chloroform

4.3 Chloroform-ethanol mixture, 3:2 by volume.

4.4 Acetone

4.5 Sulphuric acid, density 1.84 g/ml.

4.6 Nitric acid, density 1.40 g/ml.

4.7 Magnesium acetate, $Mg(CH_3COO)_2.4H_2O$, low in phosphorus.

4.8 Quinoline molybdate solution

4.8.1 Sodium molybdate

4.8.2 Distilled water

4.8.3 Citric acid

4.8.4 Quinoline, freshly distilled.

4.8.5 Dissolve 70 g of sodium molybdate, $Na_2MoO_4.2H_2O$, in 150 ml of distilled water.

4.8.6 Dissolve 60 g of citric acid in 150 ml of distilled water and add 85 ml of nitric acid.

4.8.7 Slowly pour solution (**4.8.5**) into solution (**4.8.6**), stirring constantly.

4.8.8 To 100 ml of distilled water, carefully add 35 ml of nitric acid (**4.6**) and 5 ml of quinoline (**4.8.4**). Pour this solution into solution (**4.8.7**) stirring continuously. Allow to stand for 24 hr. at room temperature. If a precipitate forms, remove it by filtration. Add 280 ml of acetone and then dilute to 1 litre with water. Keep the molybdate reagent (**4.8**) in a well-closed plastic container in a dark place.

5. Apparatus

5.1 Electrical hotplate, with magnetic stirrer.

5.2 Erlenmeyer flask, 300 ml, with reflux condenser.

5.3 Pleated filter paper, 15 cm diameter.

5.4 Volumetric flask, 250 ml.

5.5 Platinum dish, approximately 130 ml capacity.

5.6 Sintered glass filter crucible, G4.

5.7 Muffle furnace, maintained at 800°C.

5.8 Water bath

5.9 Desiccator

5.10 Erlenmeyer flask, 250 ml.

5.11 Watch-glass

5.12 Glass rod

5.13 Filter paper, ashless.

5.14 Hotplate, electrical.

5.15 Buchner flask

5.16 Drying oven, electrically heated and thermostatically controlled at 260 ± 20°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 Separation of phospholipids

6.2.1 Weigh (to the nearest 10 mg) 12-13 g of the well-mixed sample (m_0) into a 300 ml Erlenmeyer flask (**5.2**).

6.2.2 Add 100 ml of chloroform and 75 ml of ethanol to the flask, and mix thoroughly using the magnetic stirrer until a homogeneous suspension is obtained. Heat for 1 hr. under reflux with continuous stirring.

6.2.3 Allow the flask to cool and stand overnight. Filter the contents of the flask through a pleated filter paper, previously moistened with chloroform-ethanol mixture (**4.3**), into a 250 ml volumetric flask.

Rinse the Erlenmeyer flask and the filter with more chloroform-ethanol solvent, and add to the volumetric flask, finally diluting with the same solvent to 250 ml.

6.2.4 Pipette 100 ml of the solution (**6.2.3**) into a platinum dish, cover with an ashless filter paper and evaporate off the solvent cautiously over a water bath to dryness. Add 3.5 g of magnesium acetate to the dish. Cut the filter paper into pieces and add to the contents of the dish. Cover the dish with another ashless filter paper. Calcine the residue gently over a flame and then in a muffle furnace at 800°C until a white powder is obtained (about 1 hr.).

6.2.5 Dissolve the ash (**6.2.4**) carefully in 15 ml of nitric acid (by allowing the acid to flow along a glass rod) and transfer to a 250 ml Erlenmeyer flask. Rinse the dish several times with water, adding the rinsings to the flask. Dilute the flask contents to 50 ml and allow to cool to room temperature.

6.2.6 Add 50 ml of quinoline molybdate reagent (**4.8**) to the flask with continual stirring. Cover the flask with a watch-glass and boil on the hotplate for 1 min. Allow the flask to cool to room temperature, stirring 2-3 times.

6.2.7 Heat a sintered glass filter crucible (**5.6**) at 260 ± 20°C for 30 min, cool in a desiccator and weigh to the nearest mg (m_1).

6.2.8 Transfer the precipitate (**6.2.6**) to the sintered glass filter crucible with gentle suction, and wash five times with 20 ml volumes of water.

6.2.9 Dry the crucible and contents at 260 ± 20°C in the drying oven for 1 hr., cool in a desiccator and weigh to the nearest mg (m_2).

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

8.1 The lipid phosphoric acid (lecithin) content, expressed in terms of P_2O_5 as a percentage by mass of the sample (i.e. in g P_2O_5/100 g sample) is given by:

$$\% \text{ lipid phosphoric acid content} = \frac{2.5 \times (m_2 - m_1) \times 0,03207 \times 100}{m_o}$$

Where:

m_0 is the weight of sample taken;

m_1 is the weight of the empty sintered glass filter crucible;

m_2 is the weight of the sintered glass filter crucible and precipitate.

8.2 The egg-yolk content, expressed as a percentage by mass of the sample (ie in g/100 g) is given by:

% egg-yolk content (g/100 g) = % lipid phosphoric acid content × 102

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, r, deduced from the collaborative trial data summarised below (Table 1). When analysing mayonnaise containing about 5% egg-yolk, the value of r may be taken as 0.6%. This precision corresponds to a relative standard deviation of repeatability (coefficient of variance of repeatability), RSD_r, of 4-5%

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 egg-yolk contents of about 5%, R may be taken as 0.7%. This precision corresponds to a relative standard deviation of reproducibility (coefficient of variance of reproducibility), RSD_R, of about 5%.

A3 Trueness (Bias)

The collaborative trial established satisfactory precision parameters for the method. Comparison in Table 1 between the observed mean and the calculated recipe values of egg-yolk content suggests satisfactory accuracy. The mean observed values differ by no more than 0.1 g/100 g from the "expected" values.

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 0.6% egg-yolk 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 egg-yolk content of each sample was also calculated from its recipe.

Table 1 summarises the statistical data; the egg-yolk content is expressed as a percentage by mass of the sample.

TABLE 1

Statistical Analysis of the % Egg-yolk in Mayonnaise Samples

Sample	1/3	4/6	2/5
Number of laboratories retained after eliminating outliers	18	19	15
Number of laboratories eliminated as outliers	2	1	5
Number of results accepted	36	38	30
LEVEL OF ANALYTE			
"Expected" recipe value	4.7	5.1	5.6
Mean observed value	4.6	5.1	5.5
REPEATABILiTY			
Standard Deviation S_r	0.26	0.28	0.11
Relative Standard Deviation RSD_r(%)	5.7	5.5	2.0
Repeatability r [2.8 x S_r]	0.74	0.79	0.31
REPRODUCIBILITY			
Standard Deviation S_R	0.30	0.28	0.13
Relative Standard Deviation RSD_R(%)	6.5	5.5	2.4
Reproducibility r [2.8 x S_R]	0.85	0.78	0.37

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