

MAFF VALIDATED METHODS FOR THE ANALYSIS OF FOODSTUFFS

No. V8

ACIDITY IN COCOA AND CHOCOLATE PRODUCTS

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1. Scope and Field of Application

The method allows the determination of the acidity of cocoa butter.

2. Definition

Acidity: the content of acid (calculated as oleic acid) as determined by the method specified.

3. Principle

The fat sample is dissolved in a neutral mixture of ethanol and diethyl ether; the free fatty acids then in solution are titrated against standard ethanolic potassium hydroxide solution and calculated as oleic acid.

4. Reagents

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

4.1 Standard potassium hydroxide solutions

Potassium hydroxide solutions, 0.1 mol/l and 0.5 mol/l in 95% (V/V) ethanol, are stored in brown or yellow glass bottles furnished with rubber stoppers and then decanted for use. Each solution should be colourless or straw yellow. Stable colourless solutions can be prepared by either of the following procedures.

4.1.1 Solution in distilled solvent

Reflux 1 litre of ethanol with 8 g of potassium hydroxide and 5 g of aluminium pellets for one hr., then distil immediately. Dissolve the required amount of potassium hydroxide in the distillate. Allow the whole to stand for several days and decant off the clear and colourless supernatant liquid from the deposited potassium carbonate.

4.1.2 Solution in undistilled solvent

Add 4 ml of aluminium to 1 litre of ethanol and allow the mixture to stand for several days. Decant off the supernatant liquid and dissolve therein the necessary amount of potassium hydroxide. After allowing to stand 1 or 2 days, it may be used as such.

The required amount of potassium hydroxide is 5.61 g for a 0.1 mol/l solution and 28.05 g for a 0.5 mol/l solution.

4.2 Phenolphthalein, 1% (m/V) in 95% (V/V) ethanol.

4.3 Diethyl ether/ethanol mixed solvent, equal volumes of diethyl ether and 95% (V/V) ethanol containing 0.3 ml of phenolphthalein solution (4.2) in 100 ml. Neutralise immediately before use by means of 0.1 mol/l ethanolic KOH solution (4.1).

5. Apparatus

5.1 Volumetric equipment

5.2 Analytical balance

6. Procedure

Weigh (to the nearest 0.01 g) 5 - 10 g fat into a 250 ml conical flask, and add 50 - 150 ml of the mixed solvent (4.3) to dissolve the fat. Titrate, while stirring, the fat solution against the ethanolic potassium hydroxide solution 0.5 mol/l (or 0.1 mol/l for acidities less than 2%) using phenolphthalein as indicator. The end point occurs when a pink colour remains for at least ten seconds.

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 acidity, expressed as a percentage of oleic acid, is given by:

$$\% \text{ acid content} = \frac{a \times M \times 282}{10 \times m_o}$$

Where:

m_o is the mass in grams of the fat sample taken;

a is the number of millilitres of ethanolic potassium hydroxide needed for neutralisation;

M is the strength, in mol/l, of ethanolic potassium hydroxide used;
the number 282 is the molecular weight of oleic acid.

9. References

- 9.1** HJ Judd, MD Percival and R Wood, J. Assoc. Publ. Analysts, 1984, **22**, 81- 101.
- 9.2** "Determination of the acid value (A.V.) and acidity", International Union of Pure and Applied Chemistry, Standard Methods for the Analysis of Oils, Fats and Derivatives, 6th Edition, method 2.201.
- 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 V.0 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). At acidities corresponding to 1-2.5% oleic acid, r may be taken as 0.08%. This precision corresponds to a relative standard deviation of repeatability (coefficient of variance of repeatability), RSD_r , of 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). For acidities within the range 1-2.5%, R may be taken to be 0.3-0.8%, corresponding to a relative standard deviation of reproducibility (coefficient of variance of reproducibility), RSD_R , of 11%.

A3 Trueness (Bias)

The collaborative trial included the analysis of a sample of cocoa butter before and after spiking with 1% of stearic acid. An overall mean difference of 0.65% was found, suggesting a recovery of 65%, but no correction should be made.

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% acid 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 pair (W/Y) was solvent-extracted cocoa butter.

Table 1 summarises the statistical data; the acidity was calculated as oleic acid and expressed as a percentage by mass of the sample.

TABLE 1

Statistical Analysis of Acidity expressed as the % of Oleic Acid in Cocoa Butter Sample

Sample	T/Z	V/X	W/Y
Number of Laboratories retained after eliminating outliers	13	18	16
Number of Laboratories eliminated as outliers	5	0	2
Number of results accepted after eliminating outliers	26	36	32
LEVEL OF ANALYTE			
Mean observed value $\bar{x}$	1.68	1.03	2.48
REPEATABILITY			
Standard Deviation S_r	0.018	0.029	0.018
Relative Standard Deviation RSD_r (%)	1.1	2.8	0.7
Repeatability r [$2.8 \times S_r$]	0.05	0.08	0.05
REPRODUCIBILITY			
Standard Deviation S_R	0.046	0.11	0.28
Relative Standard Deviation RSD_R (%)	2.7	10.7	11.3
Reproducibility R [$2.8 \times S_R$]	0.13	0.32	0.78

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