



METHOD

DETERMINATION OF FREE FATTY ACIDS, COLD METHOD

1. SCOPE AND FIELD OF APPLICATION

This method describes the determination of free fatty acids in olive oils and olive pomace oils. The content of free fatty acids is expressed as acidity, calculated as the percentage of oleic acid.

2. PRINCIPLE

A sample is dissolved in a mixture of solvents and the free fatty acids present are titrated using a potassium hydroxide or sodium hydroxide solution.

3. REAGENTS

All the reagents should be of recognized analytical quality and the water used either distilled or of equivalent purity.

3.1. Diethyl ether; 95 % ethanol (v/v), mixture of equal parts by volume.

Neutralize precisely at the moment of use with the potassium hydroxide solution (3.2), with the addition of 0.3 ml of the phenolphthalein solution (3.3) per 100 ml of mixture.

Note 1: Diethyl ether is highly inflammable and may form explosive peroxides. Special care should be taken in its use.

Note 2: If it is not possible to use diethyl ether, a mixture of solvents containing ethanol and toluene may be used. If necessary, ethanol may be replaced by propanol-2.

3.2. Potassium hydroxide or sodium hydroxide, titrated ethanolic or aqueous solution, c(KOH) about 0.1 mol/l or, if necessary, c(KOH) about 0.5 mol/l. Commercial solutions are available.

The exact concentration of the potassium hydroxide solution (or sodium hydroxide solution) must be known and checked prior to use. Use a solution prepared at least five days before use and decanted into a brown glass bottle with a rubber stopper. The solution should be colourless or straw coloured.

If phase separation is observed when using aqueous solution of potassium hydroxide (or sodium hydroxide), replace the aqueous solution by an ethanolic solution.

Note 3: A stable colourless solution of potassium hydroxide (or sodium hydroxide) may be prepared as follows. Bring to the boil 1 000 ml of ethanol or water with 8 g of potassium hydroxide (or sodium hydroxide) and 0.5 g of aluminium shavings and continue boiling under reflux for one hour. Distil immediately. Dissolve in the distillate the required quantity of potassium hydroxide (or sodium hydroxide). Leave for several days and decant the clear supernatant liquid from the precipitate of potassium carbonate (or sodium carbonate). The solution may also be prepared without distillation as follows: to 1 000 ml of ethanol (or water) add 4 ml of aluminium butylate and leave the mixture for several days. Decant the

supernatant liquid and dissolve the required quantity of potassium hydroxide (or sodium hydroxide). The solution is ready for use.

- 3.3. Phenolphthalein, 10 g/l solution in 95 to 96 % ethanol (v/v), alkali blue 6B or thymolphthalein, 20 g/l solution in 95 to 96 % ethanol (v/v). In the case of strongly coloured oils, alkali blue or thymolphthalein shall be used.

4. APPARATUS

Usual laboratory equipment including:

- 4.1. Analytical balance;
- 4.2. 250 ml conical flask;
- 4.3. 10 ml burette class A, graduated in 0.05 ml, or equivalent automatic burette.

5. PROCEDURE

- 5.1. Preparation of the test sample

When the sample is cloudy, it should be filtered.

- 5.2. Test portion

Take a sample depending on the presumed acidity in accordance with the following table:

Expected acidity (oleic acidity g/100g)	Mass of sample (g)	Weighing accuracy (g)
0 to 2	10	0.02
> 2 to 7.5	2.5	0.01
> 7.5	0.5	0.001

Weigh the sample in the conical flask (4.2).

- 5.3. Determination

Dissolve the sample (5.2) in 50 to 100 ml of the previously neutralized mixture of diethyl ether and ethanol (3.1).

Titrate while stirring with the 0.1 mol/l solution of potassium hydroxide (3.2) (see Note 4) until the indicator changes (the colour of the coloured indicator persists for at least 10 seconds).

Note 4: *If the quantity of 0.1 mol/l potassium hydroxide solution required exceeds 10 ml, use the 0.5 mol/l solution or change the sample mass according to the expected free acidity and the proposed table.*

Note 5: *If the solution becomes cloudy during titration, add enough of the solvents (3.1) to give a clear solution.*

Carry out a second determination only if the first result is higher than the specified limit for the category of the oil.

6. EXPRESSION OF RESULTS

Acidity, as percentage of oleic acid by weight is equal to:

$$V \times c \times \frac{M}{1000} \times \frac{100}{m} = \frac{V \times c \times M}{10 \times m}$$

where:

V = the volume of titrated potassium hydroxide solution used, in millilitres;

c = the exact concentration in moles per litre of the titrated solution of potassium hydroxide used;

M = 282 g/mol, the molar mass in grams per mole of oleic acid;

m = the mass of the sample, in grams.

Oleic acidity is reported as follows:

(a) to two decimal places for values from 0 up to and including 1;

(b) to one decimal place for values from 1 up to and including 100.

PRECISION VALUES OF THE METHOD

Analysis of the collaborative test results

More than 20 laboratories took part in the collaborative test arranged by the International Olive Council in 2014 and 2015.

The test was performed on 5 samples in 2014 and 3 samples in 2015:

PA 1-2014: crude olive pomace oil

PA 2-2014: refined olive pomace oil

PA 3-2014: refined olive oil

PA 4-2014: extra virgin olive oil (Mario Solinas prize) – 2011 crop

PA 5-2014: extra virgin olive oil (Mario Solinas prize) – 2014 crop

PA 6-2015: 70% lampante olive oil + 30% grape seed oil

PA 7-2015: late harvest extra virgin olive oil

PA 8-2015: 90% lampante olive oil + 10% palm olein

The results of the collaborative tests have been statistically processed according to the rules laid down in the international standards ISO 5725-2. Outliers were examined by applying Cochran's and Grubbs' test to the laboratory results for each determination (replicates *a* and *b*) and each sample.

The table lists:

n	number of participating laboratories
outliers	number of laboratories with outlying values
mean	mean of the accepted results (g/100g)
S_r	repeatability standard deviation (g/100g)
RDS_r (%)	relative standard deviation for repeatability (S _r x 100/mean)

- r** value below which the absolute difference between two single independent test results obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time may be expected to lie with a probability of 95% (S_r multiplied by 2.8) (g/100g)
- S_r** reproducibility standard deviation (g/100g)
- RSD_r (%)** relative standard deviation for reproducibility ($S_r \times 100/\text{mean}$)
- R** value below which the absolute difference between two single test results obtained with the same method on identical test material in different laboratories with different operators using different equipment may be expected to lie with a probability of 95% (S_r multiplied by 2.8) (g/100g)

Acidity (oleic acidity g/100g) – 2014 Collaborative study					
sample	PA1	PA2	PA3	PA4	PA5
mean	6.280	0.110	0.068	0.128	0.153
N	22	22	22	22	22
outliers	1	1	2	2	0
S_r	0.052	0.007	0.006	0.004	0.007
RSD_r (%)	0.8%	6.1%	9.3%	3.2%	4.8%
R	0.144	0.019	0.018	0.011	0.021
S_R	0.191	0.027	0.015	0.019	0.036
RSD_R (%)	3.0%	24.2%	22.7%	14.7%	23.3%
R	0.535	0.074	0.043	0.053	0.100

Acidity (oleic acidity g/100g) – 2015 Collaborative study			
sample	PA6	PA7	PA8
mean	1.413	0.496	0.692
N	20	20	20
outliers	2	3	2
S_r	0.005	0.006	0.008
RSD_r (%)	0.4%	1.3%	1.1%
R	0.015	0.018	0.022
S_R	0.043	0.026	0.030
RSD_R (%)	3.1%	5.3%	4.4%
R	0.121	0.074	0.085