INTERNATIONAL

OLIVE

COUNCIL

COI/T20/Doc. no. 19/Rev. 2
2008

ENGLISH
Original: ITALIAN

METHOD OF ANALYSIS

SPECTROPHOTOMETRIC INVESTIGATION IN THE ULTRAVIOLET

FOREWORD

Spectrophotometric examination in the ultraviolet can provide information on the quality of a fat, its state of preservation and changes brought about in it by technological processes.

The absorption at the wavelengths specified in the method is due to the presence of conjugated diene and triene systems. These absorptions are expressed as specific extinctions $E_{1cm}^{1\%}$ (the extinction of 1% solution of the fat in the specified   solvent, in a thickness of 1 cm)

 conventionally indicated by K (also referred to as "extinction coefficient").

1. SCOPE

The method describes the procedure for performing a spectrophotometric examination of fats in the ultraviolet.

2. PRINCIPLE OF THE METHOD

The fat in question is dissolved in the required solvent and the extinction of the solution is then determined at the specified wavelengths with reference to pure solvent. Specific extinctions are calculated from the spectrophotometer readings.

3. EQUIPMENT

3.1.   A spectrophotometer for measuring extinction in the ultraviolet between 220 and 360 nm, with the possibility of reading individual nanometric units.

3.2.   Rectangular quartz cuvettes, with covers, having an optical length of 1 cm. When filled with water or other suitable solvent the cuvettes should not show differences between them of more than 0.01 extinction units.

3.3.   25 ml graduated flasks.

3.4.   Chromatography column having a length of 450 mm and a diameter of 35 mm with a discharge tube of diameter approximately 10 mm.

4. <u>REAGENTS</u>

4.1. Spectrophotometrically pure iso-octane (2,2,4-trimethylpentane). With reference to distilled water this should have a transmittance of not less than 60% at 220 nm and not less than 95% at 250 nm,

or

- spectrophotometrically pure cyclohexane: with reference to distilled water this should have a transmittance of not less than 40% at 220 nm and not less than 95% at 250 nm, or

- another suitable solvent capable of completely dissolving the fat.

5. <u>PROCEDURE</u>

5.1. The sample in question must be perfectly homogeneous and without suspended impurities. Oils which are liquid at ambient temperature are to be filtered through paper at a temperature of approximately 30°C, hard fats are to be homogenized and filtered at a temperature of not more than 10°C above the melting point.

5.2. Weigh accurately 0.25 g of the sample so prepared into a 25 ml graduated flask, make up to the mark with the solvent specified and homogenize. The resulting solution must be perfectly clear. If opalescence or turbidity is present filter quickly through paper.

5.3. Fill a cuvette with the solution obtained and measure the extinctions at an appropriate wavelength between 232 and 276 nm, using the solvent used as a reference.

The extinction values recorded must lie within the range 0.1 to 0.8. If not, the measurements must be repeated using more concentrated or more dilute solutions as appropriate.

5.4. When a determination of specific extinction is required after passage over alumina, proceed as follows. Place 30 g of basic alumina in suspension in hexane in the chromatography column. After the adsorbent has settled remove the excess hexane down to approximately 1 cm above the top of the alumina.

Dissolve 10 g of the fat, homogenized and filtered as described in 5.1, in 100 ml of hexane and pour the solution into the column. Collect the eluate and evaporate off all the solvent under vacuum at a temperature below 25°C.

Proceed immediately as specified in 5.2 using the fat so obtained.

5.5.   The maximum wave length of 268 nm or 270 nm shall be selected depending on whether the solvent used is iso-octane or cyclohexane, respectively.

6.   <u>**EXPRESSION OF THE RESULTS**</u>

6.1.   Record the specific extinctions (extinction coefficients) at the various wavelengths calculated as follows:

$$K_\lambda = \frac{E_\lambda}{c.s}$$

where:

K_λ :   specific extinction at wavelength $_\lambda$;

E_λ :   extinction measured at wavelength $_\lambda$;

c :   concentration of the solution in g/100 ml;

s :   thickness of the cuvette in cm.

The results are to be expressed to two decimal places.

6.2.   Spectrophotometric analysis of olive oil in accordance with the official method in the EEC regulations involves determination of the specific extinction in iso-octane solution at wavelengths of 232 and 270 nm and the determination of the variation of the specific extinction ($\triangle$ K), which is given by:

$$\triangle K = K_m - \frac{K_{m-4} + K_{m+4}}{2}$$

where K_m is the specific extinction at wavelength m, the wavelength for maximum absorption around 270 nm.

NOTE: Maximum wave lengths of 268 nm and 232 nm if iso-octane is used, and of 270 nm and 232 nm if cyclohexane is used.

APPENDIX I

Calibration of the spectrophotometer

A.2.   The equipment must be checked at intervals (at least every six months) for both wavelength response and the accuracy of the response.

A.2.1.   The wavelength may be checked using a mercury vapour lamp or by means of suitable filters.

A.2.2.   In order to check the response of the photocell and the photomultiplier proceed as follows: weigh 0.2000 g of pure potassium chromate for spectrophotometry and dissolve in 0.05N potassium hydroxide solution in a 1000 ml graduated flask and make up to the mark. Take precisely 25 ml of the solution obtained, transfer to a 500 ml graduated flask and dilute up to the mark using the same potassium hydroxide solution.

Measure the extinction of the solution so obtained at 275 nm, using the potassium hydroxide solution as a reference. The extinction measured using a 1 cm cuvette should be 0.200 ± 0.005.

PRECISION VALUES OF THE METHOD

Analysis of the collaborative test results

The precision values of the method are given in the table overleaf.

Nineteen laboratories holding IOOC recognition at the time took part in the collaborative test arranged by the Executive Secretariat in 1999. The laboratories were from eight countries.

The test was performed on five samples:

A: extra virgin olive oil
B: virgin olive oil + refined sunflower oil
C: virgin olive oil + refined olive-pomace oil
D: virgin olive oil + refined soybean oil + refined sunflower oil
E: refined olive oil + refined olive-pomace oil + refined soybean oil + lampante virgin olive oil

The results of the collaborative test organised by the IOOC Executive Secretariat have been statistically processed according to the rules laid down in the international standards ISO 5725 **Accuracy (trueness and precision) of measurement methods and results.** 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

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   Repeatability standard deviation

RDS_r (%)   Repeatability coefficient of variation (S_r x 100/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	Reproducibility standard deviation
RDS_R (%)	Reproducibility coefficient of variation (S_R x 100/mean)

Absorbency in Ultra-Violet

- at 270 nm

	A	B	C	D	E
n	17	18	18	18	18
outliers	3	0	1	0	5
mean	0.110	0.253	0.202	0.212	0.561
r	0.006	0.009	0.012	0.015	0.018
Sr	0.002	0.004	0.004	0.006	0.007
RSDr (%)	1.961	1.431	2.283	2.691	1.191
R	0.016	0.026	0.021	0.031	0.020
S_R	0.006	0.009	0.008	0.011	0.007
RSD_R(%)	5.052	3.770	3.872	5.470	1.302

-Delta K

	A	B	C	D	E
n	17	18	18	18	18
outliers	1	2	0	1	1
mean	0.000	0.020	0.010	0.003	0.040
r	-	0.000	0.003	0.002	0.007
Sr	-	0.001	0.001	0.001	0.003
RSDr (%)	-	7.720	17.920	25.660	6.421
R	-	0.004	0.006	0.004	0.017
S_R	-	0.002	0.002	0.002	0.006
RSD_R(%)	-	8.271	37.021	50.201	14.210

Normative references

ISO 5725-1: 1994   Accuracy (trueness and precision) of measurement methods and results – Part 1: General principles and definitions

ISO 5725-2: 1994   Accuracy (trueness and precision) of measurement methods and results – Part 2: Basic method for the determination of the repeatability and reproducibility of a standard measurement method

ISO 5725-5: 1994   Accuracy (trueness and precision) of measurement methods and results – Part 5: Alternative methods for the determination of the precision of a standard measurement method

ISO 5725-6: 1994   Accuracy (trueness and precision) of measurement methods and results – Part 6: Use in practice of accuracy values