Method COI T.20 Doc. 33
Fatty acids analysis in olive oils

Method of fatty acids analysis COI T20 Doc 33

What is required of a method of analysis of fatty acids in olive oils:

- Analyse as accurately as possible the fatty acid composition of any genuine olive oil.

-To be able to determine the trans isomers of fatty acids.

- Being able to determine the fatty acids of fraudulent oils that include other types of oils

- Serve to determine the composition of fatty acids in triglycerides to be used in the calculation of ECN42 and Coherence..

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The combination of ISO 5508, IOC. T 20 Doc. 17 and Doc. 24 was the tool for the analysis of fatty acids in olive oils in the IOC until 2015. At the same time, Annex X (A and B) of the EEC 2568/91 regulation compiled all these methods within of the EU

From that moment, given that the ISO 5508 standard had been withdrawn and replaced by the ISO-12966 series of standards, method T20 Doc. 33 was approved by the IOC.

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In these years of application of the method, some deficiencies in the method have been revealed, which must be solved.

-The current wording does not include the correspondence of the chromatogram peaks with fatty acids, which was detailed in the disappeared method COI T.20 Doc 24.

-The current method when it is used to apply it to the determination of the ECN42 does not work, for oils with high acidity.

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From September 2020, when I proposed a new wording in this same forum, it was first approved within the IOC chemicals group, but later, after a consultation within the EU experts, several sections of the method were disputed. In early 2021 it was referred to the IOC Methods Restricted Group. After going through the restricted group of IOC methods, in autumn 2021 a working draft was reached.

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The neutralization step indicated in point 7.1 of the method COI T.20 Doc 17 (withdrawn) should be included.

6. Reagents

6.1. Carrier gas: Pure helium or hydrogen, gas chromatography quality (caution: see appendices).

6.2. Auxiliary gas: air and hydrogen, gas chromatography quality (caution: see appendices).

6.3. Reference samples: methyl esters of pure fatty acids, in particular *cis* and *trans* isomers of octadecenoic **(1),** octadecadienoic **(2)** and octadecatrienoic **(1)** acid.

6.4. n-hexane (caution: see appendices).

7. Procedure

7.1. Preparation of the methyl esters: Use a procedure that involves basic catalysis.

Fats and oils with a free acidity of more than 3 % should be neutralised beforehand.

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Or better, neutralization can be done according to the 2-glyceryl

Monopalmitate method as indicated in Annex X of Regulation 2568/91 prior to 2015.(withdrawn)

▼M2

6. SPECIAL CASE — DETERMINATION OF TRANS-ISOMERS

It is possible to determine the content of trans-isomers in fatty acids with a number of carbon atoms between 10 and 24 by separating the methyl esters using gas chromatography capillary columns having a specific polarity.

6.1. A capillary column made of silica having an internal diameter of between 0,25 mm and 0,32 mm and a length of 50 m, coated with cyanopropisilicon, the thickness of the coating being between 0,1 and 0,3 µm (type SP 2380, C.P. sil 88, silor 10 and similar types).

▼M21

6.2. The methyl esters are prepared using procedure B set out in Annex XB. Fatty substances having a free acidity over 3 % must first be neutralised in accordance with point 5.1.1 of Annex VII.

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Fatty acids analysis in ECN42 method

Direct purification of high acidity oils does not work, presumably because free fatty acids saturate the silica in the SPE cartridge. The neutralization step for analysis of trans isomers should be used also to prepare the oil prior to column purification required by the ECN42 method to analyze fatty acids.

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As this proposals change the method, a validation of the new method should be considered looking for oils that cannot be analyzed with the current method.

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Oil category	Analytical parameter to be determined	
	No purification	Purification
Virgin olive oil with acidity ≤ 2,0% Refined Olive Oil Olive oil composed of refined oil and virgin olive oils Olive pomace oil	1. Fatty acid composition 2. 2 Trans fatty acids	1. Determination of the difference between actual and theoretical content of triacylglycerols with ECN42 2. Coherence of TAG composition with the fatty acid composition
Virgin olive oil with acidity > 2,0% Crude olive pomace oil		1. Fatty acid Composition 2. Trans fatty acids 3. Determination of the difference between actual and theoretical content of triacylglycerols with ECN42 4. Coherence of TAG composition with the fatty acid composition

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COI/T.20/Doc. No 23/ Rev. 1 2017 Neutralization step

Oils with a free acidity below 3% do not require neutralization prior to silica gel column chromatography. Oils with a free acidity above 3% do require neutralization as described in the procedure

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COI/T.20/Doc. No 23/ Rev. 1 2017 Neutralization step

Into a 1000 mL separating funnel (3.13) introduce 50 g of the oil and dissolve in 200 mL of n-hexane. Add 100 mL of isopropanol and an amount of 12% sodium hydroxide solution (4.9) corresponding to the free acidity of the oil plus 5% excess. Shake vigorously for 1 min, add 100 mL of distilled water, shake again and leave to settle. After separation, remove the bottom soap layer.

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COI/T.20/Doc. No 23/ Rev. 1
2017 Neutralization step

An intermediate layer of mucilage and insoluble matter often forms and must also be removed. Wash the hexane solution of the oil with successive 50–60 mL portions of the isopropanol/water mixture (1/1, V/V) (4.2.2) until the wash phase is neutral to phenolphthalein. Remove most of the hexane by distillation under vacuum (e.g. use a rotary evaporator) and transfer the oil to a 100 mL flask (3.5); dry it under vacuum to complete solvent removal. By the end of this procedure the acidity of the oil must be below 0.5%

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The proposals olive oils for this validation are samples

3 →5% acidity
~10 % acidity
20→30 % Acidity

In May we found four crude olive pomace oils with acidity of: 1.7%; 5.6%; 11% and 20%. We analyze fatty acids, triglycerides and acidity.

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	SPE Neut	SPE	IUPAC Neut	IUPAC	No purificat
Fatty Acids					
Mirístic C14:0	0,02	0,03	0,02	0,02	0,03
Palmític C16:0	12,71	13,35	13,23	13,07	13,04
Palmitoleic C16:1	1,18	1,18	1,14	1,17	1,19
Margaric C17:0	0,11	0,09	0,09	0,12	0,11
Margaroleic C17:1	0,14	0,14	0,13	0,15	0,15
Estearic C20:0	2,90	3,04	2,94	2,89	2,91
Oleic C18:1	68,69	68,06	68,29	68,53	68,19
Oleic (trans) C18:1 Trans	0,13	0,07	0,05	0,08	0,05
Linoleic C18:2	12,24	12,08	12,31	12,24	12,36
Linolenic C18:3	0,84	0,83	0,80	0,79	0,87
linoleic + linolenic Trans	0,93	0,05	0,03	0,09	0,13
Araquidic C20:0	0,51	0,54	0,48	0,46	0,50
Gadoleic C20:1	0,31	0,32	0,28	0,28	0,30
Behenic C22:0	0,22	0,23	0,21	0,20	0,22
Erucic C22:1	0,02	0,01	0,00	0,01	0,01
Lignoceric C24:0	0,10	0,10	0,08	0,08	0,12
Δ ECN 42	0,14	0,22	0,20	0,32	0,45
Theoretical	0,8174	0,7934	0,7882	0,7795	0,85
HPLC	0,95	1,01	0,98	1,09	1,3
Acidity	20º		20º		20

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	SPE Neut	SPE	IUPAC Neut	IUPAC	No purificat
Fatty Acids					
Mirístic C14:0	0,02	0,02	0,02	0,02	0,02
Palmític C16:0	12,86	13,05	13,08	13,09	13,07
Palmitoleic C16:1	0,92	0,91	0,89	0,91	0,93
Margaric C17:0	0,14	0,14	0,14	0,14	0,14
Margaroleic C17:1	0,22	0,22	0,21	0,23	0,22
Estearic C20:0	2,89	2,97	2,90	2,89	2,86
Oleic C18:1	70,09	69,99	70,10	70,08	69,83
Oleic (trans) C18:1 Trans	0,06	0,06	0,04	0,04	0,03
Linoleic C18:2	10,87	10,70	10,78	10,80	10,94
Linolenic C18:3	0,79	0,77	0,75	0,76	0,79
linoleic + linolenic Trans	0,04	0,04	0,03	0,02	0,03
Araquidic C20:0	0,54	0,56	0,53	0,49	0,52
Gadoleic C20:1	0,36	0,37	0,34	0,33	0,35
Behenic C22:0	0,20	0,21	0,19	0,19	0,19
Erucic C22:1	0,01	0,01	0,00	0,01	0,01
Lignoceric C24:0	0,10	0,10	0,08	0,07	0,11
Δ ECN 42	0,23	0,25	0,25	0,2100	0,23
Theoretical	0,6496	0,6232	0,6145	0,6233	0,66
HPLC	0,87	0,87	0,86	0,83	0,88
Acidity	11º		11º		11º

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	SPE Neut	SPE	IUPAC Neut	IUPAC	No Purificat
Fatty Acids					
Mirístic C14:0	0,02	0,02	0,02	0,02	0,02
Palmític C16:0	11,55	11,59	11,57	11,58	11,58
Palmitoleic C16:1	0,84	0,83	0,82	0,83	0,84
Margaric C17:0	0,06	0,06	0,06	0,06	0,06
Margaroleic C17:1	0,10	0,10	0,08	0,10	0,10
Estearic C20:0	2,92	2,94	2,90	2,89	2,85
Oleic C18:1	71,72	71,77	71,90	71,85	71,70
Oleic (trans) C18:1 Trans	0,04	0,04	0,03	0,02	0,02
Linoleic C18:2	10,79	10,69	10,72	10,75	10,85
Linolenic C18:3	0,76	0,75	0,73	0,74	0,79
linoleic + linolenic Trans	0,03	0,03	0,02	0,02	0,02
Araquidic C20:0	0,55	0,56	0,54	0,54	0,52
Gadoleic C20:1	0,35	0,35	0,32	0,33	0,35
Behenic C22:0	0,24	0,24	0,23	0,23	0,23
Erucic C22:1	0,00	0,01	0,00	0,00	0,01
Lignoceric C24:0	0,09	0,09	0,09	0,08	0,10
Δ ECN 42	0,44	0,51	0,47	0,45	0,45
Theoretical	0,6199	0,6056	0,5946	0,6035	0,64
HPLC	1,06	1,11	1,06	1,05	1,09
Acidity	5,6º		5,6º		5,6º

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	SPE Neut	SPE	IUPAC Neut	IUPAC	No Purificat
Fatty Acids					
Mirístic C14:0	0,02	0,02	0,02	0,01	0,02
Palmític C16:0	11,42	11,39	11,38	11,41	11,38
Palmitoleic C16:1	0,84	0,83	0,81	0,82	0,85
Margaric C17:0	0,06	0,06	0,06	0,06	0,06
Margaroleic C17:1	0,11	0,11	0,11	0,11	0,10
Estearic C20:0	2,83	2,85	2,82	2,80	2,77
Oleic C18:1	74,36	74,44	74,58	74,53	74,40
Oleic (trans) C18:1 Trans	0,03	0,02	0,02	0,02	0,01
Linoleic C18:2	8,44	8,38	8,39	8,43	8,54
Linolenic C18:3	0,75	0,74	0,72	0,73	0,77
linoleic + linolenic Trans	0,02	0,02	0,01	0,01	0,02
Araquidic C20:0	0,54	0,54	0,53	0,52	0,50
Gadoleic C20:1	0,33	0,33	0,31	0,31	0,32
Behenic C22:0	0,22	0,22	0,21	0,21	0,21
Erucic C22:1	0,01	0,00	0,00	0,00	0,00
Lignoceric C24:0	0,08	0,08	0,06	0,06	0,08
Δ ECN 42	0,27	0,28	0,35	0,30	0,29
Theoretical	0,4546	0,4454	0,4345	0,4423	0,47
HPLC	0,72	0,72	0,78	0,74	0,76

Acidity	1,7º	1,7º	1,7º

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As you can see there are some differences between the results of the oils purified with SPE and IUPAC column, with and without neutralization. But they were not significant. To find the cause of this agreement between all the results, we analyzed the oils again but this time without the silica purification step and we found that there were no noticeable differences either.

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This made us think that the oils had not suffered oxidation.
We concluded that the effort to do the ring test was not justified. Perhaps the shortage of oils in this harvest has removed older oils from the market that would have given significance to the exercise.

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We concluded that the effort to do the ring test was not justified. Perhaps the shortage of oils in this harvest has removed older oils from the market that would have given significance to the validation ring-test.

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In August we found a crude olive pomace oil with 31% acidity, we analyzed it, but starting with the unpurified sample. Again we found that it met the parameters of the standard, so we avoided carrying out further analysis

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	No Purificat
Fatty Acids	
Mirístic C14:0	0,04
Palmític C16:0	14,04
Palmitoleic C16:1	1,36
Margaric C17:0	0,10
Margaroleic C17:1	0,22
Estearic C20:0	2,07
Oleic C18:1	67,69
Oleic (trans) C18:1 Trans	0,07
Linoleic C18:2	12,63
Linolenic C18:3	0,71
linoleic + linolenic Trans	0,01
Araquidic C20:0	0,46
Gadoleic C20:1	0,38
Behenic C22:0	0,21
Erucic C22:1	0,00
Lignoceric C24:0	0,10
Δ ECN 42	0,45
Theoretical	0,78
HPLC	1,23
Acidity	31,10

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We will continue looking for oils to find differences that justify the ring-test

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Thank you for your attention