METHOD FOR THE EVALUATION OF THE COHERENCE OF TAG COMPOSITION WITH THE FATTY ACID COMPOSITION

1. SCOPE

The method is used to check the coherence of the triacylglycerol composition of olive oils with its fatty acid composition. The theoretical triacylglycerol composition calculated from the fatty acid composition is similar in olive oil to the experimental triacylglycerol composition. The method only indicates if the olive oil triacylglycerol composition is coherent or non-coherent.

2. PRINCIPLE

The experimental triacylglycerol (TAG) composition is compared with the theoretical obtained from the analysis of fatty acid methyl esters (FAME). The oil is purified by solid phase extraction (SPE) on silica gel cartridges. The TAG composition is determined by reverse phase high-resolution liquid chromatography (RP-HPLC) using a refractive index detector and propionitrile as the mobile phase. FAME are prepared from purified oil by methylation with a cold solution of KOH in methanol and then the esters are analysed by capillary gas chromatography using high polar columns (COI/T.20/Doc. no. 33 rev. 1). The theoretical TAG composition is calculated from the fatty acid composition by a computer program assuming a 1,3-random, 2-random distribution of fatty acids in the triacylglycerol, with restrictions for saturated fatty acids in the 2-position. The calculation method is a modification of the procedure described in COI/T.20/Doc. no. 20. Several mathematical algorithms are calculated from theoretical and experimental TAG compositions, and the resulting values are compared with those contained in a database built from genuine olive oils.

3. MATERIAL AND REAGENTS

3.1. Oil purification

3.1.1. 25-ml conical flasks.

3.1.2. 5-ml screw top glass tubes and caps fitted with PTFE joint.
3.1.3. Silica gel cartridges, 1 g (6 ml), for solid phase extraction.
3.1.4. n-Hexane, analytical grade.
3.1.5. Diethyl ether, analytical grade.
3.1.7. n-Heptane, analytical grade.
3.1.8. Acetone, analytical grade.

3.2. **HPLC analysis of triacylglycerols**
3.2.1. Micro syringes (50 µL) and needles for HPLC injection.
3.2.2. Propionitrile, super purity or HPLC grade, used as mobile phase.
3.2.3. HPLC column (25 cm x 4 mm i.d.), packed with RP-18 phase (4 µm particle size).

3.3. **Preparation of fatty acid methyl esters (See method for the determination of fatty acid methyl esters by gas chromatography; COI/T.20/Doc. no. 33 rev. 1).**
3.3.1. Methanol containing not more than 0.5% water.
3.3.2. n-heptane, analytical grade.
3.3.3. A 2N solution of potassium hydroxide in methanol. Dissolve 1.1 g of potassium hydroxide in 10 ml of methanol.
3.3.4. 5-ml screw top glass tubes and caps provided with PTFE joint.

3.4. **GC analysis of FAMEs (See method for the determination of fatty acid methyl esters by gas chromatography; COI/T.20/Doc. no. 33 rev. 1).**
3.4.1. Micro syringes (5 µL) and needles for GC injection.
3.4.2. Hydrogen or helium as carrier gas.
3.4.3. Hydrogen and air for FID detector.
3.4.4. Nitrogen or helium as auxiliary carrier gas.
3.4.5. Fused silica capillary column (50-60 m x 0.25 – 0.30 mm i.d.) coated with cyanopropylpolysiloxane or cyanopropylphenylsiloxane phases (SP-2380 or similar) with 0.20-0.25 µm of film thickness.

4. **APPARATUS**

4.1. Vacuum apparatus for solid phase extraction.

4.2. Rotary evaporator.

4.3. **HPLC equipment composed of:**
4.3.1. Degasser for the mobile phase.
4.3.2. Rheodyne injector valve or automatic injector with a 10 µL loop.
4.3.3. High-pressure pump unit.
4.3.4. Thermostatic oven for the HPLC column capable of maintaining sub-ambient temperatures (15-20 °C), (for example, Peltier type).
4.3.5. Refractive index detector.
4.3.6. Computerised data acquisition system provided with an integration program.

4.4. **Capillary gas chromatography equipment described in COI/T.20/Doc. no. 33, provided with:**
4.4.1. Split injector.
4.4.2. Flame ionisation detector (FID).
4.4.3. Oven with programmable temperature.
4.4.4. Computerised data acquisition system provided with an integration program.

4.5. **Computer with Microsoft EXCEL program.**
5. **ANALYTICAL PROCEDURE**

5.1. **Oil purification**

An SPE silica gel cartridge (3.1.3) is placed in a vacuum elution apparatus (4.1) and washed under vacuum with 6 ml of hexane (3.1.4). The vacuum is released to prevent the column from drying and a conical flask (3.1.1) is placed under the cartridge. A solution of the oil (0.12 g, approximately) in 0.5 ml of hexane (3.1.4) is loaded into the column and the solution is pulled through and then eluted with 10 ml of the solvent mixture (3.1.6) of hexane-diethyl ether (87:13 v/v) under vacuum. The eluted solvent is homogenised and approximately half of the volume is poured into another conical flask (3.1.1). Both solutions are separately evaporated to dryness in a rotary evaporator (4.2) under reduced pressure at room temperature. For triacylglycerol analysis, one of the residues is dissolved in 1 ml of acetone (3.1.8) (See first paragraph of 5.2) and poured into a 5-ml screw top glass tube. The other residue is dissolved in 1 ml of n-heptane (3.1.7) and poured into a second 5-ml screw top glass tube for preparing the fatty acid methyl esters.

*Note:* Oil purification may be done using a silica gel column, as described in IUPAC method 2.507.

5.2. **HPLC analysis of triacylglycerols**

Set up the HPLC system, maintaining the column temperature at 20°C and using propionitrile (3.2.2) as the mobile phase at a flow rate of 0.6 ml/min. When the baseline is stable run a solvent injection; if the base line appears disturbed in the region from 12 to 25 min, use another type of acetone or a mixture of propionitrile/acetone (25:75) to dissolve the sample.

*Note:* Some types of acetone produce disturbances of the baseline in the above-mentioned region.

Inject a 10 μl aliquot of the solution of purified oil in acetone (5%). The run takes approximately 60 min. Oven temperature and/or flow rate must be adjusted to achieve a chromatogram similar to that depicted in Figure 1 where trilinolenin (peak 1) elutes at 15.5 min and the resolutions between the pairs LLL/OLLn (peaks 1 and 2) and OLL/OOLn (peaks 4 and 5) are good.

The height of peak 2 (OLLn+PoLL) must reach at least 3% of the full scale.

5.3. **Preparation of fatty acid methyl esters.**

Add 0.1 mL of a 2N solution of potassium hydroxide in methanol to the solution of purified oil in 1 mL of n-heptane. Cap the tube and screw tight. Shake the tube vigorously for 15 seconds and leave to stratify until the upper layer becomes clear (5 minutes). The n-heptane solution is ready to be injected into the gas chromatograph. The solution may be left at room temperature for a maximum of 12 hours.

5.4. **GC analysis of fatty acid methyl esters**

The procedure described in the method for the determination of *trans*-unsaturated fatty acids must be used (COI/T.20/Doc. no. 33).

The GC system is set up at an oven temperature of 165 °C. The recommended oven temperature is isothermal at 165 °C for 10 min, then raising it to 200 °C at 1.5 °C/min. An injector temperature between 220 °C and 250 °C is recommended to minimise the formation of trans-fatty acids (See IOOC method). Detector temperature 250 °C.
Hydrogen or helium must be used as the carrier gas at a column head pressure of 130 kPa, approximately. Injection volume 1μL in split injection mode. A GC profile similar to that shown in Figure 2 must be obtained. Special attention must be paid to the resolution between C18:3 and C20:1 (the C18:3 peak must appear before the C20:1). To achieve these conditions, the initial temperature and/or the column head pressure must be optimised. Adjust the injector conditions (temperature, split ratio and volume injection) to minimise the discrimination of palmitic and palmitoleic acid.

The height of the C20:0 peak must be about 20% of full scale to quantify the trans isomers. If the C18:0 peak appears distorted, reduce the sample amount.

5. INTEGRATION OF CHROMATOGRAPHIC PEAKS

5.1. HPLC chromatogram

Figure 1 shows a typical HPLC chromatogram of the triacylglycerols of a purified olive oil. For peak integration, three baselines must be traced: the first between the start of peak 1 and the end of peak 3; the second between the start of peak 4 and the valley before peak 8; the third between the valley preceding peak 8 and the end of peak 18. The total area is the sum of the areas of all the peaks (identified and not identified) from peak 1 to peak 18. The percentage of each peak is given by

\[ \text{TAG}_x \% = 100 \left( \frac{A_x}{A_T} \right) \]

The percentages have to be given to two decimal figures.

5.2. GC chromatogram

Figure 2 shows a GC chromatogram of fatty acid alkyl esters obtained from a purified olive oil. Percentages of the following fatty acids must be calculated:

- Palmitic; \( P \) (C16:0) = methyl ester + ethyl ester
- Stearic; \( S \) (C18:0) = methyl ester
- Palmitoleic; \( P{o} \) (C16:1) = sum of methyl esters of the two cis-isomers
- Oleic; \( O \) (C18:1) = sum of methyl esters of the two cis-isomers + ethyl ester + trans-isomers
- Linoleic; \( L \) (C18:2) = methyl ester + ethyl ester + trans-isomers
- Linolenic; \( L{n} \) (C18:3) = methyl ester + trans-isomers
- Arachidic; \( A \) (C20:0) = methyl ester
- Eicosenoic (gondoic); \( G \) (C20:1) = methyl ester

Ethyl and trans-isomers esters may be absent in the GC chromatogram.

Total area (AT) is the sum of all the peaks appearing in the chromatogram from C14:0 to C24:0, except that corresponding to squalene. The percentage of each peak is calculated as follows:

\[ \text{FA}_x \% = 100 \left( \frac{A_x}{A_T} \right) \]

The results have to be expressed to two decimal places.

For the calculations of the computer programs, it is not necessary to normalise to 100 because this is done automatically.
**Figure 1.** HPLC chromatogram of TAGs of a “Chamlali” virgin olive oil. Main components of chromatographic peaks: (1) LLL; (2) OLLn+PoLL; (3) PLLn; (4) OLL; (5) OOLn+PoOL; (6) PLL+PoPO; (7) POLn+PPPO+PPO; (8) OOL+LnPP; (9) PoOO; (10) SLL+PLO; (11) PoOP+SPoL+SOLn+SPoPo; (12) PLP; (13) OOO+PoPP; (14) SOL; (15) POO; (16) POP; (17) SOO; (18) POS+SLS; (19) AOO.
**Table 1:** Repeatability data of the determination of virgin olive oil TAGs by HPLC at a column temperature of 20° C and using propionitrile as mobile phase.

<table>
<thead>
<tr>
<th>ECN</th>
<th>HPLC Peaks</th>
<th>TAGs</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Sample 4</th>
<th>Sample 5</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<td>RSD (%)</td>
<td>Mean (%)</td>
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<tr>
<td>42</td>
<td>1</td>
<td>LLL</td>
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<td>1.53</td>
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<td></td>
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<td>0.69</td>
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<td>5.56</td>
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<tr>
<td></td>
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<td>1.73</td>
<td>1.23</td>
<td>1.65</td>
<td>1.10</td>
<td>1.54</td>
</tr>
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</table>

\[n = 3\text{ replicates}
RSD_r = \text{Relative Standard Deviation of the repeatability}\]
Figure 2. GC chromatogram in a polar capillary column of fatty acid alkyl esters obtained from an olive-pomace oil by transesterification with a cold solution of KOH in methanol.
ANNEX I

DETERMINATION OF THE COHERENCE OF TAG COMPOSITION WITH THE FATTY ACID COMPOSITION WITH A DATA BASE BUILT FROM GENUINE OLIVE OILS

1. CALCULATION

1.1. Triacylglycerol composition

Peaks of ECN42 TAGs
LLL = peak 1
OLLn = peak 2+shoulder 2a must be summed (Note 1)
POLLn = peak 3

Note 1. In some oils, and in very good chromatographic conditions, the shoulder may be resolved as a separate peak. In this case, the area must be summed to that of peak 2.

Peaks of ECN44 TAGs
OLL = peak 4
OOLL = peak 5 + posterior shoulder, if it is present (In Figure 1, this shoulder is not present).
POLL = peak 6
POLLn = peak 7 + posterior shoulder if it is present (In Figure 1, this shoulder is not present)

Peaks of ECN46 TAGs
OLL = peak 8

1.2. Fatty acid composition

A GC chromatogram of fatty acid alkyl esters obtained by cold transmethylation with a methanolic solution of KOH from a purified olive oil is shown in Figure 2. Percentages of P, Po, S, O, L, Ln, A, and G acids must be calculated.

2. MATHEMATICAL ALGORITHMS TO CONFIRM THE COHERENCE OF THE TAG COMPOSITION OF THE OLIVE OILS.

2.1. Parameters to classify the oils in groups

LLL \_Theor\_
\Delta OOL = OOL \_Theor\_ - OOL \_HPLC\_
\Delta LLL = LLL \_HPLC\_ - LLL \_Theor\_
Ln = % linolenic acid
\Delta ECN44_p = (PLL + POLn) \_HPLC - (PLL + POLn) \_Theor

The oil sample is classified in one of the eight groups according to its \( LLL \_\text{Theor} \) value:

a) \( \leq 0.010 \% \)
b) \( > 0.010 - \leq 0.022 \% \)
c) \( > 0.022 - \leq 0.040 \% \)
d) \( > 0.040 - \leq 0.070 \% \)
e) \( > 0.070 - \leq 0.110 \% \)
f)  $> 0.110 - \leq 0.170 \%$

$g)  \, > 0.170 - \leq 0.250 \%$

$h)  \, > 0.250 - \leq 0.350 \%$

$j)  \, > 0.350 - \leq 0.550 \%$

$k)  \, > 0.550

In each group the oils are classified in subgroups according to the values of $\Delta OOL$ or $\Delta LLL$.

2.2. Parameters for comparison with the database.

2.2.1. Legal limit: $\Delta ECN42 = ECN42_{HPLC} - ECN42_{Theor}$.

The result should be within the limits include in the trade standard, although this limit is not used to verify the coherence of the TAG composition with the fatty acid methyl ester composition of the oil in the method.

2.2.2. Early criterion for all groups:

$\text{K1} = (\text{LLL}_{HPLC} + \text{OLLn}_{HPLC})^* (\text{OLLn}_{Theor} + \text{OLLn}_{Theor})^* (\text{LLL}_{HPLC} + \text{OLLn}_{HPLC})$

If $\text{K1} \leq \text{limit}_{lw}$, the oil is coherent.

If $\text{K1} > \text{limit}_{up}$, the oil is non-coherent.

2.2.3. $1^{st}$ criterion: $\Delta R3 = [\text{OLL/OLLn}]_{HPLC} - [\text{OLL/OLLn}]_{Theor}$

If $\Delta R3 \leq \text{limit}_{lw}$, the oil is coherent.

If $\Delta R3 > \text{limit}_{up}$, the oil is non-coherent.

2.2.4. $2^{nd}$ criterion: $L3 = [\Delta LLL - \Delta OLLn]/\text{OLLn}_{Theor}$

If $L3 \leq \text{limit}_{lw}$, the oil is coherent.

If $L3 > \text{limit}_{up}$, the oil is non-coherent.

Where $\Delta OLLn = \text{OLLn}_{Exp} - \text{OLLn}_{Theor}$

For group a, the $2^{nd}$ criterion is $R1_{exp} = \text{LLL}_{HPLC}/\text{OLLn}_{HPLC}$

If $R1_{exp} \leq \text{limit}_{lw}$, the oil is coherent.

If $R1_{exp} > \text{limit}_{up}$, the oil is non-coherent.

2.2.5. $3^{rd}$ criterion: $R1_{exp} = \text{LLL}_{HPLC}/\text{OLLn}_{HPLC}$

If $R1_{exp} \leq \text{limit}_{lw}$, the oil is coherent.

If $R1_{exp} > \text{limit}_{up}$, the oil is non-coherent.

For group a, the $3^{rd}$ criterion is $L3 = [\Delta LLL - \Delta OLLn]/\text{OLLn}_{Theor}$

If $L3 \leq \text{limit}_{lw}$, the oil is coherent.

If $L3 > \text{limit}_{up}$, the oil is non-coherent.

2.2.6. $4^{th}$ criterion: $\Delta R1 = [\text{LLL}/\text{OLLn}]_{HPLC} - [\text{LLL}/\text{OLLn}]_{Theor}$

If $\Delta R1 \leq \text{limit}_{lw}$, the oil is coherent.

If $\Delta R1 > \text{limit}_{up}$, the oil is non-coherent.

For groups a, b and c $\Delta R1$ only have a limit, if the value of $\Delta R1$ is lower or equal is coherent and if the value of $\Delta R1$ is higher than the limit is non-coherent.

2.2.7. $5^{th}$ criterion: $L4 = [\Delta LLL - \Delta OLLn]/\text{LLL}_{Theor}$

If $L2 \leq \text{limit}_{lw}$, the oil is coherent.

If $L2 > \text{limit}_{up}$, the oil is non-coherent.
2.2.8. **6th criterion**:

$$R_2 = \frac{[\Delta \text{OLL} \times \text{LLL}_\text{Theor}]}{[\Delta \text{LLL} \times \text{OLL}_\text{Theor}]}$$

Where $\Delta \text{LLL} = LLL_{\text{Exp}} - LLL_{\text{Theor}}$ and $\Delta \text{OLL} = OOL_{\text{Exp}} - OOL_{\text{Theor}}$

If $R_2 \geq$ limit, the oil is coherent.
If $R_2 <$ limit, the oil is non-coherent.

For groups e, f, g and h the 6th criterion also include the $\Delta \text{OOL}$. For group j the 6th criterion also include the $\Delta \text{OOL}$ and $\Delta \text{R3}$.

2.3. **Determination of limits**

For each subgroup, the limits for some parameters are given by the equation:

$$\text{limit versus } \Delta \text{ECN44p value}$$

Where $\Delta \text{ECN44p} = (PLL+POL_{\text{n}})_{\text{Exp}} - (PLL+POL_{\text{n}})_{\text{Theor}}$

The 1st, 2nd, 3rd, 4th, and 5th criteria have lower and upper limits ($\text{Limit}_{\text{Low}}$ and $\text{Limit}_{\text{up}}$), whereas the 6th criterion has only one limit. The groups a, b and c, have only 1st, 2nd and 3rd criteria with upper and lower limit and a 4th criteria with only one limit.

The values of each criterion are sequentially compared with the upper and lower limits. Values between them indicate that the next parameter has to be compared. Figure 3 shows the flow diagram of the sequential procedure.

3. **APPLICATION OF THE COMPUTER PROGRAM.**

3.1. **Load EXCEL on the computer.**

3.2. **Open the file 25AVELL_computerprogram.xlsm**

3.3. **Activate “Macro” if it is necessary.**

3.4. **Click the “Press to initiate calculation” button.**

3.5. **Enter data in yellow cells only (Figure 4)**

3.5.1. **In “Sample code”, type the description of the sample**

3.5.2. **In “Oil category” type:**

“EV” for edible virgin olive oils (extra, and virgin categories) “L” for lampante oil
“R” for the refined olive and olive (blend of virgin and refined olive oils) categories.
This code is necessary to calculate the official limit for $\Delta \text{ECN42}$.  

3.5.3. **In “Intro. FAMEs-GC”, type the fatty acid composition.**

The program automatically normalises the percentages to the sum of the eight fatty acids. If |Warning| comes up; please revise the fatty acid data.

3.5.4. **Type the triacylglycerol data in the corresponding cells. Please revise the data.**
3.6. The program calculates the theoretical triacylglycerol composition from the normalised fatty acid composition.

3.7. Using the experimental and theoretical triacylglycerol percentages, the program calculates the values of the mathematical algorithms corresponding to the oil sample and the values of the limits.

3.8. The command “Press control+letter” appears in the red band, indicating the LLI-Theor range in which the oil is classified.

3.9. To see the results, press simultaneously “control” and the specified letter keys; the results sheet for the oil sample then comes up (Figure 5).

3.10. Read the result in the red bands.
   “The oil is coherent” indicates that the oil has a TAG composition is coherent with the fatty acid composition.
   “The oil is non-coherent” indicates that the oil does not have a TAG composition coherent with the fatty acid composition and the non-coherence should be checked using other parameters.
   For each criterion, the values of the algorithms are typed in bold figures. The higher and lower limits and the values of other parameters are also indicated.

3.11. Click the button “Press to print the results” to print the results.
   Two sheets will be printed: the experimental data and the values and limits for the sample.

3.12. Click the button “Press for a new calculation” to reinitialise the program.
   All data will be erased.
Figure 3. Flow diagram of the sequential procedure
**Figure 4.** Data sheet of the calculation program
### Figure 5. Results sheet

#### Criteria for LIL theor. 0.022 - 0.040

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<td>Refined Olive Oil</td>
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<table>
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<th>Parameter</th>
<th>Value</th>
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<th>Lower Limit</th>
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#### Criterion K1

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Proceed to the Criterion 1

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<th>Lower Limit</th>
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</tbody>
</table>

Proceed to the Criterion 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Higher Limit</th>
<th>Lower Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L_3$</td>
<td>0.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Delta C_{OIL}$</td>
<td>-0.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Delta C_{N44p}$</td>
<td>0.26</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Proceed to the Criterion 3

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Higher Limit</th>
<th>Lower Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_1$</td>
<td>0.63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Delta C_{OIL}$</td>
<td>-0.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Delta C_{N44p}$</td>
<td>0.26</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Proceed to the Criterion 4

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Higher Limit</th>
<th>Lower Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta R_1$</td>
<td>0.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Delta C_{OIL}$</td>
<td>-0.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Delta C_{N44p}$</td>
<td>0.26</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The oil is coherent

Press to Print Results

Press for a New Calculation
ANNEX II
SELECTIVITY, SPECIFICITY, AND RELIABILITY OF THE METHOD

The method is based in a mathematical algorithm to determine the coherence or non-coherence of the triacylglycerol composition with the fatty acid composition, this fact can only be check as a binary test or qualitative method, and, in consequence, its statistical performance characteristics were evaluated according to qualitative algorithms. The main properties of qualitative methods are selectivity, specificity, and reliability.

Selectivity is defined as the method’s ability to produce results that are exclusively dependent on the measurand. Selectivity is quantified as the ratio between the number of true positive tests and the sum of the numbers of true positive and false-negative tests.

Specificity is defined as the ultimate level of selectivity that is the absolute absence of interferences. Specificity is formulated as the ratio between the number of true negative tests and the sum of these and the number of false-positive tests.

Reliability is the consequence of the previous analytical properties, and it is formulated as the complement to the sum of probabilities of errors of the first and second kind. The former error is defined as the ratio between the number of false positives and the total number of adulterated samples, while the latter error is the ratio between the number of false negatives and the total number of genuine samples.

Qualitative test methods are based on binary responses, 3 quantitative reference levels are involved to produce them, namely the LOD, the cut-off limit, and the threshold limit. Cut-off and threshold limits allow determination of the concentration zones of the adulterant in which the correct binary response occurs: No, below the limits; Yes, above them. Table 3 shows the values of the cited limits, estimated by implementing a series of experiments at increasing concentrations of hazelnut oils in olive oils and so determining the probability associated with the cut-off limit (8% with 94% probability). The threshold limit value was fixed at 10% because the ellipses of confidence at 94% of PCA did not include any sample spiked at 10%.

The data in table 1 shows the performance characteristics of the method calculated from the three-collaborative trial performed by the IOC.

Table 1

<table>
<thead>
<tr>
<th>Performance characteristics</th>
<th>Values, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>86</td>
</tr>
<tr>
<td>Selectivity</td>
<td>94</td>
</tr>
<tr>
<td>Detection limit</td>
<td>5</td>
</tr>
<tr>
<td>Cut-off Limit</td>
<td>8</td>
</tr>
<tr>
<td>Threshold Limit</td>
<td>10</td>
</tr>
</tbody>
</table>