

Food Technology and Biotechnology Laboratory  
Olive oil test report **44-24en** (sample code)

Date of sample receipt: 23/01/2024

Date of issue of the report: 05/02/2024

**Customer**

Name: **BRIST d.o.o.**  
Address: Trgovačka 40, 52215 Vodnjan

**Sample information**<sup>1</sup> (category declared by the customer)

Quality category: Extra virgin olive oil  
Product name: **"Brist Oleum"**; LOT 04-2023

Description of the sample and the sampling procedure:

The sample was received in one bottle made of dark glass, containing 0.5 litres. The bottle was filled with the sample to the top, and originally sealed.

<sup>1</sup> The laboratory disclaims any responsibility with regard to information received from the customer. The test results and declarations of conformity in this report refer only to the tested sample, but not to the oil from which the sample was made because the laboratory is not responsible for sampling.

**RESULTS OF THE DETERMINATION OF PHENOLIC COMPOUNDS IN OLIVE OIL BY LIQUID  
CHROMATOGRAPHY**

**Concentration of total and major hydrophilic phenols from olive oil**

Date of testing: 29.-30.01.2024.

Compound/parameter	Result (mg tyrosol / kg oil)
<b>Total phenols</b> <sup>1</sup>	<b>305 mg/kg</b>

of which:

<b>Hydroxytyrosol, tyrosol and their derivatives (secoiridoids)</b>	<b>261 mg/kg</b>
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Method of analysis: COI/T.20/Doc. No 29/Rev.2, 2022, Method 1: Determination of biophenols in olive oils by HPLC. Value of the RRF coefficient used for concentration calculation was estimated to be: 5,14.

**Mišljenje i tumačenje**

Phenolic compounds with a hydrophilic character naturally occurring in virgin olive oil (polyphenols, biophenols), although not being considered as criteria for market categorization of virgin olive oil (1), are a very important aspect of its quality. Hydrophilic phenols are responsible for the antioxidant stability or durability of the oil and exhibit numerous beneficial effects on the human body (2). The European Food Safety Authority (EFSA) issued a scientific opinion based on which the health claim was approved: "**olive oil polyphenols contribute to the protection of blood lipids from oxidative stress**". The claim may be used only for olive oil, which contains at least 5 mg of hydroxytyrosol and its derivatives (e.g., oleuropein complex and tyrosol) per 20 g of olive oil. Furthermore, information is given to the consumer that "the beneficial effect is obtained with a daily intake of 20 g of olive oil" (3).

Also, hydrophilic phenols are carriers of the positive taste properties of virgin olive oil, namely bitterness and pungency (2). It was determined that oleocanthal is the compound most responsible for the sensation of strong pungency, while the bitterness is determined by oleacein as well as by oleuropein and ligstroside aglycones (2).

By analysis of this oil sample it was determined that the sum of the concentrations of hydroxytyrosol, tyrosol and their derivatives (complex phenols from the secoiridoid group) is 261 mg/kg, which is above the lower limit for the health claim. That is, considering that the lower limit for the use of this health claim about the beneficial effect of polyphenols from olive oil is defined as a daily intake of 20 g of olive oil containing at least 5 mg polyphenols/20 g (250 mg/kg), it can be concluded that the daily intake of the recommended amount of 20 g of olive oil from which this analysed sample was taken, the stated beneficial effect on the human body can be achieved.

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Research by Kotsiou and Tasioula-Margari (2016) conducted on 42 samples of extra virgin olive oils, showed that the concentration of phenolic compounds has a wide variability in the range between 250 and 930 mg/kg. Oils with a total phenol content of about 600 are characterized as oils with a relatively high concentration of phenols, whilst oils with about 200 mg/kg, as oils with a relatively low concentration of phenols (4).

Therefore, based on the results of the analysis **this oil sample could conditionally be classified in the class of virgin olive oils with a medium concentration of phenols.**

Since hydrophilic phenols from olive oil are subject to oxidation during the storage period protecting the oil molecules from oxidation, their total concentration decreases over time, and the composition of individual phenols changes (2). It was found that during 12 months of storage, the phenol concentration decreases by 30% on average (4). The oil can be protected from oxidation if stored in containers made of inert material, protected from light and at a temperature between 14° and 24°C, in containers full to the top or best filled in atmosphere of an inert gas.

**Concentrations of individual hydrophilic phenols (phenolic profile)**

Date of testing: 29.-30.01.2024.

Compound/parameter (identification based on method of analysis)	Result (mg/kg)
<b>SIMPLE PHENOLS, total 14 mg/kg</b>	
tirosol (2)	3,4
hidroksitirosol (1)	4,2
hidroksitirozil-acetat (8)	2,8
tirosil-acetat (15)	0,7
vanilin (6)	3,3
<b>FENOLIC ACIDS, total 3 mg/kg</b>	
Vanillic acid (3) + Caffeic acid (4)	1,1
para-Coumaric acid (7)	1,7
ortho-Coumaric acid (10)	0,0
Ferulic acid (9)	0,0
Cinnamic acid (19)	0,0
<b>FLAVONOIDS, total 16 mg/kg</b>	
Luteolin (22)	9,7
Apigenin (25)	4,6
Methyl-luteolin (26)	1,3
<b>LIGNANS, total 19 mg/kg</b>	
Pinoresinol +1-Acetoxy-pinoresinol (18)	19,3

Compound/parameter (identification based on method of analysis)	Result (mg/kg)
<b>SECOIRIDOIDS, total 253 mg/kg</b>	
oluropein (13)	16,9
Oleuropein aglycone, dialdehyde form (14)	22,2
Decarboxymethyl oleuropein aglycone, oxidised dialdehyde form (11; 11a)	5,7
3,4-DHPEA-EDA <sup>2</sup> or <b>oleacein</b> (12)	60,9
dekarboksimetil ligstrozid aglikon, oksidirani dialdehidni oblik (16 i 16a)	1,9
p-HPEA-EDA <sup>3</sup> ili <b>oleocanthal</b> (17)	31,3
Oleuropein aglycone, oxidised aldehyde and hydroxylic form (21; 21a; 21b)	0,0
Oleuropein aglycone, aldehyde and hydroxylic form (23)	23,8
Ligstroside aglycone, dialdehyde form (20)	45,9
Ligstroside aglycone, oxidised aldehyde and hydroxylic form (24; 24a; 24b)	6,6
Ligstroside aglycone, aldehyde and hydroxylic form (27)	38,0

Method of analysis: COI/T.20/Doc. No 29/Rev.2, 2022, Method 1:  
Determination of biophenols in olive oils by HPLC



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Abbreviations:

<sup>1</sup> Total phenols are expressed in mg/kg of tyrosol, with syringic acid as the internal standard and the relative response factor between tyrosol and syringic acid of 5,4. Total phenols represent the sum of hydroxytyrosol, tyrosol, natural and oxidised oleuropein and ligstroside aglycone derivatives, lignans, flavonoids and phenolic acids which are individually listed in the table on page 2/3 of the report.

<sup>2</sup> 3,4-dihydroxy phenyl ethyl-alcohol linked to the dialdehyde form of decetoxy elenolic acid (other synonyms: deacetoxy oleuropein aglycone, dialdehyde form; decarboxymethyl oleuropein aglycone, dialdehyde form; decarboxymethyl elenolic acid linked to hydroxytyrosol)

<sup>3</sup> p-dihydroxy phenyl ethyl-alcohol linked to the dialdehyde form of decetoxy elenolic acid (other synonyms: deacetoxy ligstroside aglycone, dialdehyde form; decarboxymethyl ligstroside aglycone, dialdehyde form; decarboxymethyl elenolic acid linked to tyrosol)


Literature list:

- (1) COMMISSION IMPLEMENTING REGULATION (EU) 2022/2105 of 29 July 2022 laying down rules on conformity checks of marketing standards for olive oil and methods of analysis of the characteristics of olive oil
- (2) Servili M, Sordini B, Esposito S, Urbani S, Veneziani G, Di Maio I: Biological activities of phenolic compounds of extra virgin olive oil. *Antioxidants* 3: 1–23, 2014.
- (3) European Community. Council Regulation No. 432/2012 of 16 May 2012 establishing a list of permitted health claims made on foods, other than those referring to the reduction of disease risk and to children's development and health. *Off. J. Eur. Communities* 2012, L136, 1–40.]
- (4) Kotsiou K, Tasioula-Margari M: Monitoring the phenolic compounds of Greek extra-virgin olive oils during storage. *Food Chemistry*, 200:255–262, 2016.

Head of laboratory:

  
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Director:

  
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Laboratory recognised by the International Olive Council (IOC) for the physico-chemical analysis of olive oils and olive pomace oils (Type A) and for the sensory analysis of virgin olive oils (01/12/2023- 30/11/2024)

Institut za poljoprivredu i turizam



Institute of Agriculture and Tourism



Food Technology and Biotechnology Laboratory

IPT-7.8-PBL-Ob02-izd.04

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Date of sample receipt: 23/01/2024

Date of issue of the report: 06/02/2024

**Customer**

Name:	<b>BRIST d.o.o.</b>
Address:	<b>Trgovačka 40, 52215 Vodnjan</b>

**Sample information**<sup>1</sup> (category declared by the customer; parameters (1) - (8) should be tested to confirm the declared category)

Quality category:	Extra virgin olive oil
Product name:	<b>"BRIST OLEUM" LOT 04-2023</b>
Description of the sample and the sampling procedure:	The sample was received in two bottles made of dark glass, containing 0.25 and 0.5 litres. The bottles were filled with the sample to the top, and originally sealed.

<sup>1</sup> The laboratory disclaims any responsibility with regard to information received from the customer. The test results and declarations of conformity in this report refer only to the tested sample, but not to the oil from which the sample was made because the laboratory is not responsible for sampling.

**Test results**

Quality characteristic	Result ± Measurement uncertainty	Unit	Limit values for Extra Virgin Olive Oil <sup>1/2</sup>	Method
PHYSICO-CHEMICAL TESTING			Date of testing: 01-02/02/2024	
(1) Acidity	<b>0,14 ± 0,01</b>	% as oleic acid	≤ 0,80	M01 <sup>4</sup>   COI/T.20/Doc. No 34/Rev.1 2017
(2) Peroxide value	<b>5,5 ± 0,3</b>	mEqO <sub>2</sub> / kg	≤ 20,0	M02 <sup>4</sup>   COI/T.20/Doc. No 35/Rev.1 2017
(3) K <sub>232</sub>	<b>1,96 ± 0,06</b>	-	≤ 2,50	M03 <sup>4</sup>   COI/T.20/Doc. No 19/Rev.5 2019
(4) K <sub>268/270</sub>	<b>0,13 ± 0,02</b>	-	≤ 0,22	
(5) ΔK	<b>0,00 ± 0,00</b>	-	≤ 0,01	
FATTY ACID ETHYL ESTERS AND WAXES DETERMINATION			Date of testing: 29-31/01/2024	
(6) Fatty acid ethyl esters	<b>10 ± 1</b>	mg / kg oil	≤ 35	M04 <sup>4***</sup>   COI/T.20/DOC. No 28/Rev.3 2022
Waxes (C <sub>42</sub> - C <sub>46</sub> )	<b>27 ± 6</b>	mg / kg oil	≤ 150	
SENSORY TESTING			Date of testing: 06/02/2024	
(7) Median of the main defect	<b>0,0</b>	Intensity of perception on a scale from 0 to 10	= 0,0	M05 <sup>4</sup>   The International Olive Council's method for the organoleptic assessment of virgin olive oil (COI/T.20/Doc. No 15/Rev. 10 2018)
(8) Median of fruitiness "green"	<b>6,0</b>		> 0,0	
Median of bitterness	<b>5,8</b>		-	
Median of pungency	<b>6,3</b>		-	
Optional terminology for labelling purposes:	<b>"medium green fruitiness   medium bitterness   intensive pungency   well balanced oil,,</b>			

<sup>1</sup> COMMISSION DELEGATED REGULATION (EU) 2022/2104 and COMMISSION IMPLEMENTING REGULATION (EU) 2022/2105, of 29 July 2022 (both)

<sup>2</sup> IOC TRADE STANDARD APPLYING TO OLIVE OILS AND OLIVE POMACE OILS (COI/T.15/NC No 3/Rev. 18 June 2022)

<sup>4</sup> Method accredited according to ISO / IEC 17025 standard

**Statement of conformity**

<b>COMPLETE EVALUATION OF CONFORMITY</b>
According to <b>COMMISSION IMPLEMENTING REGULATION (EU) 2022/2105 of 29 July 2022 (Annex III., Table 1)</b> and <b>COMMISSION DELEGATED REGULATION (EU) 2022/2104 of 29 July 2022</b> , as well as according to the <b>Trade Standard applying to olive oils and olive pomace oils of the International Olive Council (IOC) (COI/T.15/NC No 3/Rev. 18 June 2022)</b> , olive oil sample <b>44-24</b> complies with all the quality requirements for the category <b>Extra virgin olive oil</b> .
<b>EXPLANATION</b>

The Food Technology and Biotechnology Laboratory is authorized as the official laboratory for the analysis of olive oil in official control procedures (M01 - M06).

The panel for sensory analysis of olive oil of the Institute is authorized as an official and professional panel by the Ministry of Agriculture of the Republic of Croatia (M05) and is on the list of authorized EU Panels.

\* Test(s) not included in the scope of the COI recognition for this laboratory | \*\* Test(s) not included in the accreditation field for this laboratory | \*\*\* Tests not covered by laboratory authorization





Laboratory recognised by the International Olive Council (IOC) for the physico-chemical analysis of olive oils and olive-pomace oils (Type A) and for the sensory analysis of virgin olive oils (01/12/2023- 30/11/2024)

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According to COMMISSION DELEGATED REGULATION (EU) 2022/2104 of 29 July 2022 (Annex III., Table 1.) together with COMMISSION IMPLEMENTING REGULATION (EU) 2022/2105 of 29 July 2022 (Article 2., paragraph 1., points (a) and (b)), and according to the IOC Trade Standard Applying to Olive Oils and Olive Pomace Oils (COI/T.15/NC No 3/Rev. 18 June 2022) the following declarations of conformity apply:

**COMPLIANT** - with 95% probability of coverage, the results of physico-chemical testing together with expanded measurement uncertainty, for quality parameters: **Acidity, Peroxide value, K232, K268 / 270, ΔK, Ethyl esters of fatty acids**, as well as the results of sensory testing, with CVR ≤ 20 %, for quality parameters: **Median fruitiness** and **Median of the main defect**, in this sample of olive oil are located within the limits of the category **Extra virgin olive oil**.

**COMPLIANT** - with a 95% probability of coverage, the result of the chemical testing together with the expanded measurement uncertainty for the parameter **Waxes (C<sub>42</sub> – C<sub>46</sub>)** in this olive oil sample are located within the limits of the category **Extra virgin olive oil**. Note: Other authenticity parameters in this sample have not been tested, so this declaration applies to quality parameters stated above.

**Note:** The declaration of conformity is given based on the following pre-agreed decision rules:

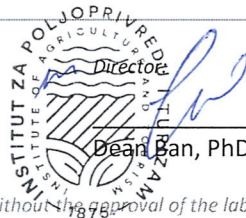
The customer requested verification of the conformity of the **quality characteristics** with the requirements for those characteristics of the quality category declared on the product as prescribed in Commission Delegated Regulation (EU) 2022/2104 of July 29, 2022 (Article 2; Annex I) and Commission Implementing Regulation (EU) 2022/2105 of July 29, 2022 (Article 9).

Therefore, the conformity decision is based on the acceptance limits selected on the basis of the "simple acceptance rule" (Eurachem/CITAC Guide, 2nd ed. 2021, available at: [www.eurachem.org](http://www.eurachem.org)) given that Article 9, paragraph 5 Commission Implementing Regulation (EU) 2022/2105 stipulates that "the results of the analysis are directly compared with the limit values".

The expanded measurement uncertainty of each parameter was calculated according to the method of analysis. It was assumed that the estimate of the measured quantity for a particular parameter has a normal probability distribution, and the specific risk was used to calculate the risk. Conformance statements are binary.

Head of laboratory:

Marina Lukić, PhD



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