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STORAGE OF EXTRA VIRGIN OLIVE OIL AND ITS IMPACT ON FATTY ACID LEVELS

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ABSTRACT

Peroxide value, acid value, and fatty acid content were monitored during two-year storage in the following products: Luccese Olio extra vergine d´oliva (LOEVDO), Blanqueta extra-virgin olive oil (BEVOO) and Agrocreta extra-virgin olive oil (AEVOO). In LOEVDO and BEVOO, peroxide value increased by 50%; AEVOO showed 80% growth. Maximum acid value was found solely in LOEVDO (<0.8%). All the analyzed samples contained almost identical proportions of fatty acids. Minute amount of lauric acid (C 12:0) was indentified in BVEOO samples only. The two-year storage resulted in growth of oleic and stearic acid levels and in decrease of linoleic and palmitic acids concentration. The percentage of SAFA, MUFA and PUFA complied with recommended levels.

Keywords: extra virgin olive oil, storage, peroxide value, acid value, fatty acids, oleic acid, SAFA, MUFA, PUFA

INTRODUCTION

Due to potential health benefits as well as to its specific organoleptic properties **(Rodrigues *et al.*, 2015)**, demand for high-quality extra-virgin olive oil has been rising recently. Quality of virgin olive oil is given by the following factors: variety, region of growing, climate, ripeness, storage period, and type of packaging. Phenolic substances enhance its nutritional, biological and sensory qualities, and its shelf life; moreover, they also improve its resistance to autooxidation (**Muco *et al.*, 2012; Albot, 2015; Kalogeropoulos and Kaliora, 2015; Koidis and Boskou, 2015)**.
More than 90% of olive oil is found in olive fruit mesocarp (**Korbelář and Endris, 1990; Lopez *et al.*, 2014)**; it contains mostly triacyl glycerols such as triglycerides fats responsible for hydrophobicity. The oil includes also small amount of free fatty acids (FFA), glycerol, phosphatides, pigments, flavourings, sterols, phenolic substances, and microscopic olive particles contributing to its uniqueness **(Boskou, 2006; Muco *et al.*, 2012; Lopez *et al.*, 2014)**.
As reported by **Albot (2015), Kalogeropoulos and Kaliora (2015), and Koidis and Boskou (2015),** olive oil decomposes in the course of its aging; quite substantial amount of oleic acid is formed, acid value grows and flavour weakens. Low acidity contributes to long shelf-life of extra-virgin olive oils. By increasing proportion of free fatty acids, oxygen exerts negative influence on sensory properties of oil. Temperatures above 25 ºC together with light accelerate its going rancid and cause decrease of antioxidant levels, too. At room temperature and in the shade, olive oil can be kept in a closed vessel for two years. After opening, oil should be consumed within a short period, since oil keeps its qualities for approximately one year and then it goes rancid.
Several studies **(Anonymous 1, 1991; Frohn, 2002; Anonymous 2, 2013; Dalmia and Perkins, 2014; Peri, 2014)** deal with quality of olive oil, factors guaranteeing its authenticity, methods employed for analysis of various oil qualities and with definition of its chemical and organoleptic properties.
According to production procedure employed and to acid content, a European directive **(Anonymous 2, 2013)** classifies olive oil into the following categories:

A) Extra-virgin olive oil. A natural cold-pressed oil without addition of chemicals or biochemical substances; it is characterized by acid value lower than 0.8%.
B) Virgin olive oil. A natural product obtained by pressing that is not treated with chemicals; its acid value is higher than 1%.
C) Olive oil. It contains mixture of refined and virgin olive oil.

Frohn (2002) and **Kotsiou and Tasioula-Margari (2016)** recommend storage of olive oil in well tighten packagings such as dark glasses or cans at temperatures ranging between 10 °C to 16 °C preferably in airy, dark, and cool rooms. **Kotsiou and Tasioula-Margari (2016)** regard places close to oven or induction cooktop the worst sites for oil storage. Permanent opening and closing oil keeping bottles and their fullness exert negative influence on oil qualities **(Piscopo and Poi, 2012)**. Bigger amounts of oil can be frozen **(Raffo *et al.*, 2015; Korifi *et al.*, 2016)**. At lower storage temperatures, oil gets thick showing soft butter consistence and it becomes turbid due to agglomerates of opaque particles. After heating it, turbidity disappears and the oil regains its properties and nutritive values **(Frohn, 2002)**.

Research on changes of physico-chemical properties of extra virgin oil stored for five month was implemented by **Salek *et al.* (2017)**; another researcher **(Orey, 2009)** studied natural effects of extra virgin oil.

The objective of our study was to monitor influence of two-year storage of selected extra virgin oils (Luccese Olio extra vergine d´oliva, Italy; Blanqueta extra virgin olive oil, Spain; and Agrocreta extra virgin olive oil, Spain) on peroxide and acid value and on content of selected fatty acids.

MATERIAL AND METHODS

Characteristics of samples

For each type of olive oil, three packagings from identical batch with identical minimum shelf-life were purchased in supermarkets. The oils were stored in a dark and dry place at 20 °C.
The following oils were subjected to the study:
Luccese Olio extra vergine d´oliva (LOEVDO) - Extra virgin olive oil (Italy); best consumed before 11/2015. This prime selection olive oil is manufactured by mechanical means directly from olive fruits.
Blanqueta extra virgin olive oil (BEVOO) - Best quality extra virgin olive oil (Spain); best consumed before 10/2015. BEVOO is obtained by mechanical pressing without using chemical additives.
Agrocreta extra virgin olive oil (AEVOO) - Prime quality extra virgin olive oil (Greece); produced directly from olive fruits by mechanical processing; best consumed before 10/2015.
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Determination of peroxide value and acid value

Peroxide value (meq $O_2.kg^{-1}$) specifies amount of oxygen that is able to oxidize iodide to iodine and Acid value shows amount of KOH ($mg.g^{-1}$) essential for neutralization of free fatty acids. Both of these parameters were determined according to the literature methodology **(Chakrabarti, 2003).**

GC Analyses of FAMEs

Lipids including fatty acids (FAs) are considered as essential human nutrients that can be classified according to absence or presence of unsaturated bonds into the following categories: saturated fatty acids (SFAs), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA).
Human body can produce both SFAs and MUFAs, but essential fatty acids (EFAs), a subcategory of PUFAs with first double bond located at C3 or C6, cannot be synthesized by humans **(Grofová, 2010).**
Fatty acids were determined by gas chromatography (GC) via their methyl esters (FAMEs) in lipid extracts obtained using the method described hereafter without drying. Solution of sodium hydroxide in methanol (0.5 M, 4 ml) was added to lipid extract obtained from 2g of a sample into a 250 ml flask. The flask was closed with a stopper and heated under nitrogen atmosphere in a heating block (LTHS 250, Brněnská Drutěva, Brno, Czech Republic). As a methylation agent, freshly prepared 15% solution of boron trifluoride in methanol (5 mL) was added then. After two minutes, heptane (15 mL) and saturated solution of sodium chloride (2 mL) were added and the sample was removed from the heating block. After adding heptane (15 mL) and saturated solution of sodium chloride (40 mL) to extract FAMEs, the phases were agitated and then subsequently separated and washed with saturated solution of sodium hydroxide (40 mL). The heptane phase was separated and anhydrous sodium sulphate was added then.
Determination of FAMEs content was implemented via a Shimadzu GC-2010 gas chromatograph equipped with flame ionization detector (FID) and a HP-88 (Agilent Technologies, Englewood, Colorado, USA) capillary column (100 m × 0,25 mm, 88 % cyanopropyl- arylpolysiloxane stationary phase with film thickness of 0.25 μm). The analysis was performed under the following conditions: injection volume 1.0 μL; the temperature of injection port 250 °C with the split ratio 1: 100; nitrogen used as a carrier gas. Temperature program of the column included the undermentioned parameters: temperature 80 ° C/5 min, 200 ° C/30 min, and 250 ° C/15 min. Identification of FAMEs was conducted by comparison of their retention time with the values of 37 FAMEs contained in a reference standard. As an internal standard, methyl undecanoate was used for quantification of FAMEs. The determined FA content is expressed as a percentage of the total FAMEs amount.

RESULTS AND DISCUSSION

Table 1 shows changes of peroxide and acid values of selected extra-virgin olive oils during storage.

Table 1 Changes of Peroxide value (meq $O_2.kg^{-1}$) and Acid value (mg $KOH.g^{-1}$) in selected extra-virgin olive oils during storage (mean±S.D.).

	meq $O_2.kg^{-}$			mg $KOH.g^{-1}$		
	LOEVDO	BEVOO	AEVOO	LOEVDO	BEVOO	AEVOO
10/2014	1.49±0.12	1.36±0.08	4.07±0.18	0.69±0.08	0.65±0.10	0.79±0.12
4/2015	1.58±0.18	1.51±0.12	4.97±0.22	0.70±0.10	0.80±0.08	1.02±0.22
1/2016	2.23±0.19	2.61±0.21	5.19±0.38	0.79±0.19	0.90±0.17	1.54±0.31

Caption: LOEVDO - Luccese Olio extra vergine d´oliva; BEVOO - Blanqueta extra virgin olive oil; AEVOO - Agrocreta extra virgin olive oil

Peroxide value increased by 50% in LOEVDO and BEVOO and by 80% in AEVOO. The EU 299/2013 directive specifies maximum peroxide values for individual types of olive oil in the following way: extra-virgin olive oil ≤20 meq O_2/kg and olive pomace oil ≤15 meq O_2/ kg. For practical reasons, the acid value is now expressed as the acidity of the oil, which is the amount of free fatty acids to the total amount of fatty acids in % (**AOCS Official Method, 2009**). The so-called acidity can be calculated from the acid number. It is recommended to keep acidity of extra-virgin olive oil below <0.8%. Only acidity of LOEVDO did not go beyond the maximum recommended value. **Muco *et al.* (2012)** suggest that acidity in virgin olive oil should not exceed 2%≤. Acidity in all the studied samples fulfilled the above limit.

Fourteen fatty acids (Table 2) were detected during two-year storage in extra-virgin olive oils.

Table 2 Percentage of fatty acids detected in selected extra-virgin olive oils during two-year storage

Fatty acid	LOEVDO			BEVOO			AEVOO		
Month/year	10/2014	04/2015	01/2016	10/2014	04/2015	01/2016	10/2014	04/2015	01/2016
C12:0	ND	ND	ND	0.02	0.02	0.02	ND	ND	ND
C14:0	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.01
C15:0	ND	ND	ND	ND	ND	ND	ND	ND	ND
C16:0	12.52	12.52	12.52	11.71	11.71	11.71	11.65	11.65	11.65
C16:1(cis-9)	0.90	0.90	0.90	0.69	0.59	0.59	0.68	0.68	0.68
C17:0	0.08	0.08	0.08	0.04	0.04	0.04	0.05	0.05	0.05
C17:1(cis-10)	0.13	0.13	0.13	0.06	0.06	0.06	0.08	0.08	0.08
C18:0	2.94	2.94	2.94	2.57	2.74	2.79	2.79	2.79	2.79
C18:1(trans-9)	0.02	0.02	0.02	0.02	0.02	0.03	0.04	0.04	0.04
C18:1(cis-9)	72.45	72.45	72.45	76.17	76.72	76.72	75.90	75.90	75.90
C18:2(all-cis-9,12)	9.53	9.53	9.53	7.15	7.03	7.03	7.35	7.35	7.35
C18:3(all-cis-9,12,15)	0.25	0.25	0.25	0.29	0.29	0.29	0.27	0.27	0.27
C20:0	0.42	0.42	0.42	0.42	0.41	0.41	0.47	0.47	0.47
C20:1(cis-11	0.75	0.75	0.75	0.74	0.74	0.73	0.73	0.73	0.73

Caption: LOEVDO - Luccese Olio extra vergine d´oliva; BEVOO - Blanqueta extra virgin olive oil; AEVOO - Agrocreta extra virgin olive oil

All the analyzed samples contained almost identical proportion of individual fatty acids; small amount of lauric acid (C 12:0) was found solely in BVEOO.
Oleic acid (C18:1 cis-9) showed to be the most abundant fatty acid; its percentage exceeded 70% in all the samples. Lesser amounts of the following acids were detected in the analyzed samples: heptanoic, heptadecanoic, arachidic, gondoic, and palmitoleic acid. These acids play important role in characterisation of individual cultivars, as reported by **Muco *et al.* (2012)**.
A legislative directive **(Anonymous 1, 1991)** specifies maximum content of myristic (0.1%), linolenic acid (0.9%), arachidonic (0.7%), eicosanic (0.5%), behenic (0.3%), and lignoceric (0.5%) acids in extra-virgin olive oil. During two-year storage, no sample showed levels of myristic (0.1%), linolenic (0.9%) or eicosanic (0.5%) acids exceeding the aforementioned limits. Neither behenic nor lignoceric acids were detected.

Table 3 shows percentage of SAFA, MUFA and PUFA in selected extra-virgin olive oils

Table 3 Percentage of SAFA, MUFA and PUFA in selected extra-virgin olive oils (mean±S.D.)

	ΣSAFA	ΣMUFA	ΣPUFA
		%	
Luccese Olio extra vergine d´oliva	15.99±1.02	74.29±2.15	9.78±0.86
Blanqueta extra virgin olive oil	14.78±0.99	77.68±2.43	7.55±0.69
Agrocreta extra virgin olive oil	14.97±0.84	77.43±2.19	7.62±0.61

Captions: SAFA - Saturated Fatty Acids; MUFA- Mono Unsaturated Fatty Acids; PUFA – Poly Unsaturated Fatty Acids

The levels of the above fatty acids are in agreement with findings **(Anonymous 1, 1991; Frohn, 2002; Muco *et al.*, 2012; Anonymous 2, 2013; Dalmia and Perkins, 2014; Peri, 2014)** reported by various researchers who detected levels of SAFA ranging between 8-25%, contents of MUFA between 55-85% and amounts of PUFA between of 4-20%.

CONCLUSION

Investigation of selected extra-virgin olive oils Olio extra vergine d´oliva (LOEVDO), Blanqueta extra-virgin olive oil (BEVOO) and Agrocreta extra-virgin olive oil (AEVOO) stored for two years showed significant changes of their properties. Peroxide value grew by 50% in LOEVDO and BEVOO; AEVOO demonstrated even 80% increase, which proves its limited stability. Solely LOEVDO did not exceed the maximum acid value (<0.8%). All the samples showed almost identical percentage of monitored fatty acids; BVEOO contained minute amount of lauric acid (C 12:0). During the storage, increase of oleic and stearic acids content was found and decrease of linoleic and palmitic acids levels was observed. SAFA, MUFA and PUFA percentage is in compliance with recommended values.

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