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Natural enrichment of refined rapeseed oil with phenols and chlorophylls from olive leaves of Oblica cultivar

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ABSTRACT

Olive leaves as by-products of olive farming are rich natural source of bioactive compounds with antioxidant properties. In this study, the efficiency of the natural maceration of fresh and steam blanched olive leaves in refined rapeseed oil was investigated, as well as the effect of degree of leaf fragmentation (whole, cut, ground) and maceration time on transfer of bioactive compounds (polyphenols) and pigments (chlorophylls) in oil. In oils obtained by maceration, changes of total phenolic compounds and total chlorophylls were determined spectrophotometrically. The effect of these oil preparation procedures on oil quality indicators was also investigated through free fatty acid content and spectrophotometric indices. The content of total phenols and chlorophylls increased in oils obtained by maceration of fresh and steam blanched olive leaves, and were in statistically significant correlation with leaf fragmentation degree. The highest content of total phenols was achieved in oils with whole fresh leaves (220.4 mg/kg) after seven days of maceration while the chlorophylls transfer to oils was the most efficient when ground steam blanched leaves were macerated for 28 days (79.10 mg/kg). Maceration of olive leaves slightly deteriorated the quality of refined rapeseed oil, equally in oils with fresh and steam blanched olive leaves. This simple preparation procedure can be efficiently used for enrichment of refined oils with natural antioxidants.

Introduction

Olive leaves are a highly valuable by-product of olive farming and processing. Considerable amount of olive leaves is obtained by pruning of olive trees (approximately 6.25 kg of fresh leaves per tree) while in olive oil industry leaves can present 10% of the total harvested olives mass (Talhaoui et al., 2015). This cheap and unexploited source of energy and natural bioactive compounds is mostly disposed of as a vegetable waste or is burnt. Olive leaves are rich in antioxidants, particularly in phenols and colouring pigments (chlorophylls and carotenoids) which can be recovered and exploited for food, pharmaceutical and cosmetic products (Souilem et al., 2017). Olive leaves contain important quantities of phenolic compounds (15-70 mg/g fresh mass) which are compositionally very similar to those in olive fruits and products thereof (Rahmanian et al., 2015; Souilem et al., 2017). Phenols in olive leaves are mainly secoiridoids in form of aglycones or glucosides (oleuropein and ligstroside) and flavonoids (rutin, quercetin, taxifolin, apigenin, luteolin) followed by the simple phenols (hydroxytyrosol, tyrosol, caffeic acid) (Abaza et al., 2017). They possess a strong antioxidant capacity (Benavente-Garcia et al., 2000), broad antimicrobial activity and several health benefits such as anti-inflammatory, cardioprotective, antidiabetic effect (Özcan and Matthaus, 2017) and play beneficial role

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in gut microbiota (Žugčić et al., 2019). Therefore, olive leaf extracts rich in phenols can be used for the enrichment of various food products and as natural food additives. The addition of olive leaf extracts and their hydrolysates to refined oils can replace synthetic antioxidants like butylated hydroxyanisole and butylated hydroxytoluene. Olive leaf extract rich in oleuropein and hydroxytyrosol improved the oxidative stability and quality of refined olive and olive pomace oil (Bouaziz et al., 2010) and other refined vegetable oils (Jimenez et al., 2011) and extended usage life of pan-frying refined oils (Zribi et al., 2013). The oxidative stability improvement of refined oils was also achieved by the addition of chlorophyll pigments extracted from olive leaves (Jaber et al., 2012). Moreover, olive leaves can be directly added to olives during the olive oil extraction (Malheiro et al., 2013; Malheiro et al., 2017) while promising enrichment of olive oil with oleuropein derivatives was attained by the addition of olive leaves extract during the malaxation of olive paste (Kiritsakis et al., 2017) leading to improved oil sensory attributes.

To our knowledge, limited attempts have been made to enrich oil matrix straight from the plant material (Nenadis et al., 2010). Therefore, in this research we investigated the efficiency of natural maceration of fresh and dried steam blanched olive leaves (cv. Oblica) in refined rapeseed oil, as well as the effect of leaves fragmentation degree (whole, cut, ground) and maceration time on transfer of hydrophilic phenols and pigments (chlorophylls) in oil. The effect of these oil preparation procedures on the oil quality indicators was also monitored.

Materials and methods

Olive leaves and preparation of oil samples

Olive leaves of Oblica cultivar were collected during the pruning in olive orchard located in Lun (the island of Pag) in March 2018. Only sound and undamaged leaves were torn off from olive branches by hand. Selected leaves were washed in a distilled water four times and left to dry on a filter paper. Morphological characteristics of Oblica leaves were determined according to the International Olive Council methodology (IOC, 1997). One-half of the leaves was used in fresh state while another half was dried at 40 °C to a constant mass in the drying oven.

Three forms (whole, cut and ground) of fresh or dried olive leaves were prepared for maceration in oil. Leaves were cut lengthwise in stripes of approximate width 2-3 mm. Each of three forms of oven dried olive leaves was separately steamed during 10 min in the steam cooking pot. A filter paper was inserted on the metal grid of steam cooker to reduce loss of plant material. After steaming, each fraction was dried at room temperature. Each of three forms of fresh and dry steamed leaves were weighted in dark bottles, which were filled with refined rapeseed oil purchased on the Croatian market. All leaf-oil preparations contained 15% (m/m) of Oblica leaves and were well shaken before maceration at room temperature in dark place. Oil preparations with fresh olive leaves were macerated seven consecutive days, while oil preparations with dry steamed olive leaves were macerated for 14, 21 and 28 days. Leaf-oil preparations were prepared in triplicate for each time point in the experiment and each of three forms of leaves (whole, cut, ground). At the end of maceration time, leaf-oil preparations were filtered through a rough filter paper. In order to facilitate filtration of oil preparations with ground leaves, they were centrifuged at 3000 rpm (Electra Medical Corporation, MA, USA) for 3 min just before filtration. All analytical determinations were done on the same day when oils were separated from the olive leaves.

Determination of dry mass and moisture content in olive leaves

Dry mass (d. m.) and the moisture content in olive leaves were determined gravimetrically after drying at 40 °C to a constant mass. Determinations were done in 11 replications.

The analysis of total phenolic content in olive leaves and oil samples

Phenolic compounds from olive leaves were extracted and determined using Folin-Ciocalteu assay according to Marinova et al. (2005). Ground dried olive leaves (0.5 g) were extracted with 25 mL of methanol 80% (V/V) on an ultrasonic bath for 20 min. An aliquot (10 mL) of the extract was centrifuged for 5 min at 4000 rpm and 200 μL of clarified extract was used in Folin-Ciocalteu assay. The absorbance was measured at 750 nm (HACH spectrophotometer DR/400, CO, USA) against a blank solution. The total phenolic content in Oblica leaves were determined from a caffeic acid calibration curve and expressed in mg per g of dry mass. Phenolic compounds from oils were extracted by liquid-liquid extraction and determined using Folin-Ciocalteu reagent according to Koprivnjak et al. (2010).

Determination of chlorophyll content in oil samples

The chlorophyll content, expressed as mg of pheophytin "*a*" per kg of oil, was determined spectrophotometrically at 630, 670 and 710 nm following the procedure of Pokorny et al. (1995).

Quality parameters of oil samples

Free fatty acids (FFA) and spectrophotometric indices (K232 and K268) were determined in oil samples according to the analytical methods described in European Commission Regulation EEC 2568/91 (EEC, 1991).

Statistical analysis

Experimental data were statistically tested by a One-way ANOVA analysis of variance. The homogeneity of variance was tested by the Brown–Forsythe test. The mean values were compared by Tukey's honest significant difference test. The correlations among the content of bioactive compounds, quality indices, maceration time and leaf fragmentation degree were determined by calculating the Pearson's coefficients. The results were considered statistically significant at $p < 0.05$. All statistical analyses were performed using the software package Statistica 13 (StatSoft, Inc., Tulsa, OK, USA).

Results and discussion

Refined rapeseed oil was selected as oil matrix for the enrichment with bioactive compounds from olive leaves due to its stability and high oleic acid content i.e., due to similar fatty acid composition as virgin olive oil (EEC 1991; NN 11/2019). Morphological characteristics of leaves used in the study (average leaf length 5.7 cm, average width 1.5 cm and elliptic shape of leaf, Table 1) are consistent with characteristics of Oblica leaves (Strikić et al., 2007). Olive leaves of Oblica cultivar contained 64.1% of dry mass and 27.8 mg/g d. m. of total phenols (Table 1) and can be considered a rich source of phenols (Souilem et al., 2017). Putnik et al. (2017) determined a higher content of total phenols in Oblica leaves (53.2 mg/g d. m.) using pressurised liquid extraction technique. Oil preparations with fresh olive leaves were macerated for shorter time (up to seven days) to avoid fermentation of fresh plant material. Dried leaves were also studied as olive pruning generates large quantities of leaves that can be stabilized by drying and as such stored until utilization. Dried olive leaves were steam blanched for 10 min since study of Stamatopoulus et al. (2012) confirmed this pre-treatment improves oleuropein extractability from 25 to 35 times in ethanol-water mixtures. Moreover, our preliminary results showed that transfer of phenols from non-blanched dried olive leaves into oil was very low: oils macerated for 21 days with steam blanched leaves contained four times higher content of total phenols than oils with non-blanched leaves (data not shown). Maceration of dried steam blanched leaves lasted up to 28 days in order to allow longer leaf-oil contact and transfer of bioactive compounds in rapeseed oil.

A statistically significant transfer of phenols from fresh leaves into oils was detected immediately after one day of maceration at all three degrees of leaf fragmentation (Table 2). The highest increase of total phenolic content was observed in oils with ground fresh leaves (from 2.0 mg/kg to 64.5 mg/kg) while the increase was modest in oils with whole fresh leaves (from 2.0 mg/kg to 26.3 mg/kg) on the first day of treatment. Phenol transfer into oils was gradual during seven days of maceration and higher in oils with whole and cut fresh leaves compared to oils with ground leaves, especially from the third to the seventh day of maceration. A total phenolic content in oils with fresh leaves was in a strong statistically positive correlation with maceration time and significantly negative in relation to a leaf fragmentation degree (Table 5A). Lower total phenolic content in oils with fresh ground leaves compared to whole and cut leaves starting from the third maceration day could be due to a higher availability of substrates for endogenous polyphenol oxidase in ground form of fresh leaves (Ortega-Garcia et al., 2008). Enrichment with phenols was the most efficient when whole fresh leaves were used (220.4 mg/kg), representing, however, a transfer of only 8% total phenolic content from fresh olive leaves to oils. This total phenolic content is comparable to those commonly found in virgin olive oils (40 to 1000 mg/kg) (Servili et al., 2004). Nenadis et al. (2010) achieved a similar effect in enrichment of virgin olive oils by the addition of 15% olive leaves. Oils macerated with dried steam blanched leaves, although macerated for a longer period (28 days), contained fivefold lower total phenolic content than oils with fresh leaves. Irrespective of a leaf fragmentation degree and the duration of maceration, a total phenolic content in these oils was low (37.3 – 44.9 mg/kg) compared to oils with fresh olive leaves. This result could be attributed to a leaching effect during steam blanching and to a heat treatment (Stamatopoulus et al., 2012).

Table 1. Morphological characteristics, water content, dry mass and mass fraction of total phenols in olive leaves (Oblica cv.)

Leaf length (cm)[1]	Leaf width (cm) [1]	Length/width[1]	Water content (%)[2]	Dry mass (d. m. %)[2]	Total phenols (mg/g d. m.) [3]
5.7 ± 0.8	1.45 ± 0.2	3.95 ± 0.7	35.9 ± 1.7	64.1 ± 1.7	27.8 ± 0.9

[1] *Results are means of 100 determinations ± standard deviation.*
[2] *Results are means of 11 determinations ± standard deviation.*
[3] *Mass fraction of total phenols is expressed as mg caffeic acid/g leaves d. m. Results are means of three analytical determinations ± standard deviation.*

Table 2. Total phenolic content (mg/kg of oil) in refined rapeseed oils after maceration of fresh and dry steam blanched olive leaves (whole, cut, ground)

Type of leaves	Maceration time (days)	Total phenols mass fraction (mg caffeic acid/kg of oil)[1]		
		Oil with whole leaves	Oil with cut leaves	Oil with ground leaves
fresh	0[2]	2.0 ± 0.1 ^{a}	2.0 ± 0.1 ^{a}	2.0 ± 0.1 ^{a}
	1	26.3 ± 0.9 $^{b, A}$	50.5 ± 3.9 $^{b, B}$	64.5 ± 4.7 $^{b, C}$
	2	59.7 ± 2.1 $^{c, A}$	88.2 ± 3.7 $^{c, B}$	85.6 ± 6.0 $^{bc, B}$
	3	102.1 ± 3.8 $^{d, A}$	138.8 ± 4.0 $^{d, B}$	73.0 ± 4.1 $^{b, C}$
	4	148.8 ± 1.3 $^{e, A}$	135.3 ± 10.2 $^{d, A}$	81.2 ± 5.4 $^{bc, B}$
	5	181.7 ± 2.5 $^{f, A}$	158.7 ± 6.8 $^{d, A}$	100.2 ± 18.6 $^{c, B}$
	6	201.1 ± 11.3 $^{g, A}$	183.8 ± 18.4 $^{e, A}$	104.6 ± 12.6 $^{c, B}$
	7	220.4 ± 5.3 $^{h, A}$	201.7 ± 8.6 $^{e, B}$	87.6 ± 1.5 $^{bc, C}$
dried steam blanched	14	37.3 ± 0.6 $^{a, A}$	38.2 ± 1.6 $^{a, A}$	43.7 ± 3.3 $^{a, B}$
	21	39.6 ± 1.9 $^{a, A}$	39.0 ± 2.3 $^{a, A}$	44.9 ± 3.7 $^{a, A}$
	28	38.4 ± 3.5 $^{a, A}$	43.1 ± 0.2 $^{b, A}$	40.8 ± 0.5 $^{a, A}$

[1] *Results are means of three determinations (3 oil preparations × 1 analytical determination) ± standard deviation. Mean values labelled by different lowercase letters in the same column and within each type of leaves are significantly different while means labelled by uppercase letters in the same row and within each type of leaves are significantly different (Tukey's test, $p < 0.05$).*
[2] *Control sample (refined rapeseed oil).*

Among the leaf fragmentation degrees, maceration of ground fresh and dried steam blanched leaves provided an appreciable total chlorophylls transfer into oils (Table 3). A positive effect of a higher leaf fragmentation degree on a chlorophyll content in oils was also confirmed by statistically significant correlations in Tables 5A and 5B. Increased leaf-oil surface and broken leaf cell structures (as well as thylakoids with chloroplasts) by grinding enhanced chlorophylls transfer into the oil. A decrease of total chlorophyll content in oils with ground fresh leaves from the third to the seventh day of maceration could be due to the activity of various leaf enzymes, which decompose chlorophylls, such as chlorophyllase, lipoxygenase and magnesium decatalase (Yilmaz and Gökmen, 2016). A lower damage level of cut leaves resulted in significantly lower total chlorophyll content in oils after seven days of maceration than in oils with ground leaves (both in fresh and dried steamed leaves). Total chlorophyll content achieved in oils is similar to levels of total chlorophylls in virgin olive oils (2.4 – 64.1 mg/kg according to Giuliani et al., 2011). As it was expected, oils with whole fresh olive leaves contained very small amount of total chlorophylls due to undamaged leaf structure and lower leaf-oil contact surface than in oils with cut and ground leaves. Heat and steam treatment of olive leaves seemed to contribute to the enhanced chlorophyll transfer to the oil during maceration (up to 79.10 mg/kg in oil with ground leaves). This effect could be due to a heat inactivation of endogenous enzymes which oxidize and change chlorophyll structure, as well as due to a lower water activity in oils with dried steamed leaves.

Natural maceration of olive leaves slightly worsened selected indicators of refined rapeseed oil quality, equally in oils with fresh and dried steam blanched leaves (Table 4, Fig. 1, Fig. 2). Refined rapeseed oil contained 0.12% FFA. After 7 and 28 days of maceration of fresh and dried steam blanched leaves, FFA were not higher than 0.23%, what is below the permitted level for refined oils (FFA ≤ 0.3%; NN 11/2019). This moderate albeit statistically significant increase of FFA could be due to the presence of water in fresh olive leaves and increased water activity in oils. Nevertheless, there were no major differences among the three forms of macerated leaves, despite what could have been expected considering a higher water availability from smaller leaf fragments. Spectrophotometric indices (K232 and K268) are usually used for quality and authenticity assessment

of cold pressed oils such as virgin olive oil (EEC, 1991). Rapeseed oil used for the enrichment had considerably higher values of spectrophotometric indices than those stipulated for virgin olive oils (K232 ≤ 2.60; K268 ≤ 0.25; EEC, 1991). These high values are an outcome of a decolouration step in a refining process. Maceration of fresh leaves resulted in slightly higher K232 and K268 values after seven days (Fig. 1) what is confirmed by statistically significant correlations of these indices with maceration time (Table 5A). Oils with dried steam blanched leaves were macerated for 28 days what significantly increased K232 in oils with cut and ground leaves (Fig. 2). Nenadis et al. (2010) reported a similar decrease in oil quality during enrichment of virgin olive oil with olive leaves. Oxidative deterioration of oil during maceration of olive leaves may be due to oxidative enzyme activity (lipoxygenase in olive leaves) as well as to the exposure to oxygen during preparation and macerate storage.

Table 3. Total chlorophylls (mg pheophytin *a*/kg of oil) in refined rapeseed oils after maceration of fresh and dry steam blanched olive leaves (whole, cut, ground)

Type of leaves	Maceration time (days)	Total chlorophylls (mg pheophytin *a*/kg of oil)[1]		
		Oil with whole leaves	Oil with cut leaves	Oil with ground leaves
fresh	0[2]	nd[3]	nd	nd
	1	0.06 ± 0.03 $^{a, A}$	3.13 ± 0.14 $^{a, A}$	15.60 ± 6.33 $^{a, B}$
	2	0.05 ± 0.04 $^{a, A}$	3.35 ± 0.48 $^{a, A}$	23.47 ± 11.80 $^{a, B}$
	3	0.10 ± 0.01 $^{ab, A}$	4.51 ± 0.65 $^{ab, A}$	48.57 ± 8.06 $^{b, B}$
	4	0.14 ± 0.01 $^{b, A}$	4.47 ± 0.35 $^{ab, A}$	32.54 ± 8.58 $^{ab, B}$
	5	0.22 ± 0.03 $^{c, A}$	5.08 ± 1.96 $^{ab, A}$	31.41 ± 11.44 $^{ab, B}$
	6	0.24 ± 0.01 $^{c, A}$	5.28 ± 0.45 $^{ab, A}$	16.29 ± 3.79 $^{a, B}$
	7	0.35 ± 0.03 $^{d, A}$	6.65 ± 1.17 $^{b, B}$	20.32 ± 1.61 $^{a, C}$
dried steam blanched	14	0.29 ± 0.04 $^{a, A}$	39.14 ± 2.24 $^{a, B}$	73.86 ± 0.95 $^{a, C}$
	21	0.37 ± 0.04 $^{a, A}$	43.81 ± 2.05 $^{b, B}$	76.20 ± 1.50 $^{a, C}$
	28	0.48 ± 0.07 $^{a, A}$	48.23 ± 1.37 $^{c, B}$	79.10 ± 1.14 $^{b, C}$

[1] *Results are means of three determinations (3 oil preparations × 1 analytical determination) ± standard deviation. Mean values labelled by different lowercase letters in the same column and within each type of leaves are significantly different while means labelled by uppercase letters in the same row and within each type of leaves are significantly different (Tukey's test, p < 0.05).*
[2] *Control sample (refined rapeseed oil).*
[3] *Not detected.*

Table 4. Free fatty acid content (FFA, % of oleic acid) in refined rapeseed oils after maceration of fresh and dry steam blanched olive leaves (whole, cut, ground)

Type of leaves	Maceration time (days)	FFA (% of oleic acid)[1]		
		Oil with whole leaves	Oil with cut leaves	Oil with ground leaves
fresh	0[2]	0.12 ± 0.01 ^{a}	0.12 ± 0.01 ^{a}	0.12 ± 0.01 ^{a}
	1	0.17 ± 0.01 $^{b, A}$	0.20 ± 0.01 $^{bc, B}$	0.18 ± 0.01 $^{b, A}$
	2	0.23 ± 0.02 $^{cd, A}$	0.20 ± 0.01 $^{bc, A}$	0.21 ± 0.01 $^{c, A}$
	3	0.23 ± 0.01 $^{cd, A}$	0.19 ± 0.01 $^{bc, B}$	0.21 ± 0.00 $^{c, AB}$
	4	0.22 ± 0.01 $^{cd, A}$	0.20 ± 0.02 $^{bc, A}$	0.19 ± 0.01 $^{bc, A}$
	5	0.19 ± 0.01 $^{b, AB}$	0.17 ± 0.02 $^{b, A}$	0.21 ± 0.01 $^{c, B}$
	6	0.23 ± 0.01 $^{d, A}$	0.21 ± 0.01 $^{c, A}$	0.21 ± 0.02 $^{c, A}$
	7	0.20 ± 0.01 $^{bc, AB}$	0.19 ± 0.01 $^{bc, A}$	0.22 ± 0.01 $^{c, B}$
dried steam blanched	14	0.19 ± 0.02 $^{a, A}$	0.19 ± 0.01 $^{a, A}$	0.19 ± 0.01 $^{a, A}$
	21	0.18 ± 0.01 $^{a, A}$	0.20 ± 0.00 $^{a, B}$	0.19 ± 0.00 $^{a, A}$
	28	0.18 ± 0.01 $^{a, A}$	0.19 ± 0.00 $^{a, A}$	0.20 ± 0.01 $^{a, A}$

[1] *Results are means of three determinations (3 oil preparations × 1 analytical determination) ± standard deviation. Mean values labelled by different lowercase letters in the same column and within each type of leaves are significantly different while means labelled by uppercase letters in the same row and within each type of leaves are significantly different (Tukey's test, p < 0.05).*
[2] *Control sample (refined rapeseed oil).*

Table 5. Correlation coefficients of total phenolic content, total chlorophyll content, free fatty acids, spectrophotometric indices, maceration time, leaf fragmentation degree for oils with fresh olive leaves (A) and for oils with dried steam blanched olive leaves (B)

A) Oils with fresh olive leaves (n = 63)

	Total phenolic content	**Total chlorophyll content**	**FFA[1]**	**K_{232}**	**K_{268}**	**Maceration time**	**Leaf fragmentation degree**
Total phenols	1	**-0.35[2]**	0.09	**0.31**	0.20	**0.76**	**-0.37**
Total chlorophylls		1	0.01	0.04	**0.65**	0.00	**0.79**
FFA			1	0.14	0.19	0.22	-0.14
K_{232}				1	**0.47**	**0.57**	0.01
K_{268}					1	**0.54**	**0.51**
Maceration time						1	0.00
Leaf fragmentation degree							1

B) Oils with dried steam blanched olive leaves (n = 27)

	Total phenolic content	**Total chlorophyll content**	**FFA[1]**	**K_{232}**	**K_{268}**	**Maceration time**	**Leaf fragmentation degree**
Total phenols	1	**0.61[2]**	0.11	0.22	0.34	0.13	**0.60**
Total chlorophylls		1	0.31	**0.46**	**0.70**	0.06	**0.99**
FFA			1	0.20	0.16	0.05	0.29
K_{232}				1	**0.42**	**0.42**	**0.44**
K_{268}					1	0.04	**0.71**
Maceration time						1	0.00
Leaf fragmentation degree							1

[1] *free fatty acids*
[2] *correlation coefficients in bold are significant at the level of 0.05*

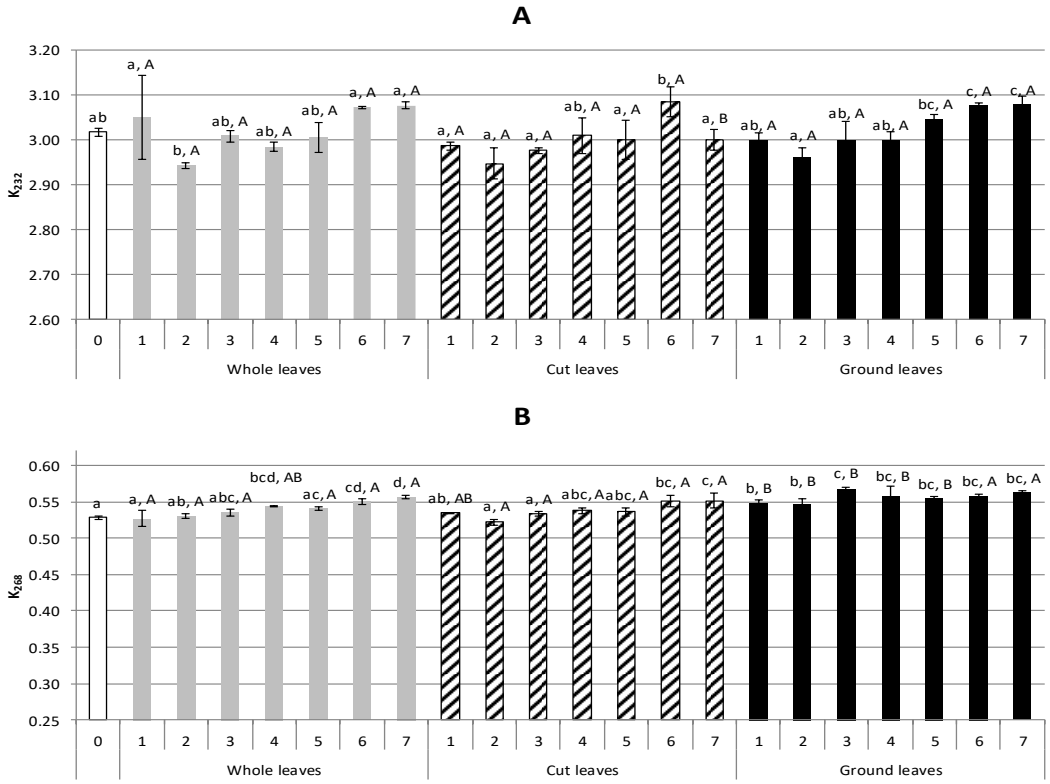

Fig 1. Spectrophotometric indices K232 (A) and K268 (B) of refined rapeseed oil after maceration of fresh olive leaves (whole, cut, ground). Results are means of three determinations (3 oil preparations × 1 analytical determination) ± standard deviation. Numbers from 1 to 7 indicate days of maceration while 0 indicates a control sample (refined rapeseed oil). Mean values labelled by different lowercase letters within each type of leaf fragmentation are significantly different while means labelled by uppercase letters within the same day of maceration are significantly different (Tukey's test, $p < 0.05$)

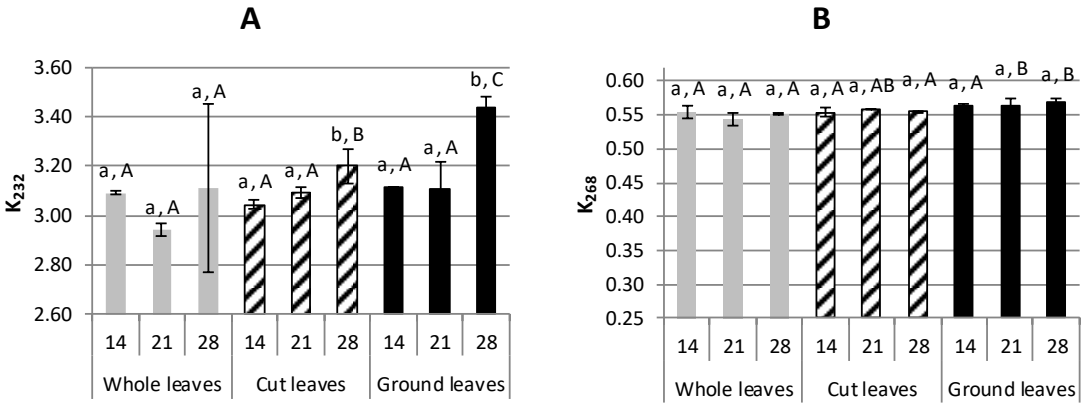

Fig 2. Spectrophotometric indices K232 (A) and K268 (B) of refined rapeseed oil after maceration of dry steam blanched olive leaves (whole, cut, ground). Results are means of three determinations (3 oil preparations × 1 analytical determination) ± standard deviation. Numbers 14, 21 and 28 indicate days of maceration. Mean values labelled by different lowercase letters within each type of leaves fragmentation are significantly different while means labelled by uppercase letters within the same day of maceration are significantly different (Tukey's test, $p < 0.05$).

Conclusion

Natural maceration of olive leaves in the refined rapeseed oil results in a considerable enrichment of oils with valuable bioactive compounds, primarily phenols and chlorophylls, up to the levels comparable to virgin olive oils. Maceration time and a leaf fragmentation degree influence the transition of these compounds in oil. Whole fresh leaves provide the highest total phenolic content in oils while ground dried steamed leaves supply oils with the highest total chlorophyll content. Although this type of enrichment slightly decreases oil quality, it represents a simple and promising strategy to enhance the nutritive value of refined oils and to create speciality oils. Moreover, the consumption of these types of products may contribute to the intake of beneficial phenolic compounds from olives.

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