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Chemical changes in extra virgin olive oils from Slovenian Istra after thermal treatment

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Abstract

Changes in the physico-chemical parameters of extra virgin olive oils after heating for 142 h at 100 °C with an air flow 10 L/h were investigated. The experimental study was carried out on the two predominant olive cultivars in Slovenian Istra – cv. *Istrska belica* and cv. *Leccino*. The data obtained showed that oils from *Istrska belica* were more stable than those from *Leccino*. Peroxide values and spectrophotometric data showed higher amounts of oxidation products in oils from *Leccino* than in those from *Istrska belica*. After thermal treatment fatty acid composition was changed more in *Leccino* oils; particularly the amounts of polyunsaturated fatty acids dropped significantly, while α-tocopherol was completely depleted in all samples. The content of total biophenols decreased from 598 mg/kg to 241 mg/kg in *Istrska belica* oils and from 391 mg/kg to 176 mg/kg in *Leccino* oils. HPLC data showed that transformation of secoiridoid biophenols to the simple biophenols, tyrosol and hydroxytyrosol took place.

Keywords: Olive oil; cv. *Istrska belica*; cv. *Leccino*; Oxidative stability; Biophenols; Thermal treatment

1. Introduction

Numerous investigations support a relationship between the Mediterranean diet and a low incidence of several diseases, such as cardiovascular diseases, cancer etc. Being the main source of fat, olive oil is an important component of this diet. One of the reasons for olive oil's health benefits is its high content of monounsaturated fatty acids. What makes olive oil distinct from other oils with high contents of monounsaturated fatty acids, such as high oleic sunflower oil, is the presence of biophenols with strong antioxidant properties. Both simple and complex biophenols were detected, with the latter being the most abundant (Nissiotis & Tasioula-Margari, 2002). Lignans and flavonoids are rather common in other foodstuffs. The olive oil lignans are pinoresinol, acetoxypinoresinol and hydroxypinoresinol (Owen et al., 2000). There are two main flavonoids – luteolin and apigenin (Pinelli et al., 2003). In contrast to these, secoiridoids are characteristic of olive biophenols. The two main secoiridoids in olive drupes are oleuropein and ligstroside. They are both present in olive oil, as well as a number of their derivatives formed during olive oil extraction and later during storage and use of oil (Rovellini & Cortesi, 2002). Besides their beneficial health effects (Owen et al., 2000) biophenols give olive oils their unique taste and they represent an important contribution to the oxidative stability of olive oil (Baldioli, Servili, Peretti, & Montedoro, 1996). The oxidative stability of virgin olive oils correlates mainly with the concentration of hydrophilic biophenols, particularly with secoiridoid biophenols containing the hydroxytyrosol moiety (Nissiotis & Tasioula-Margari, 2002). An important minor component of virgin olive oils is α-tocopherol, which protects oil from oxidation

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at elevated temperatures (Quiles, Ramírez-Tortosa, Gómez, Huertas, & Mataix, 2002).

Oxidation is the most important cause of oil and fat deterioration. The primary lipid oxidation products are hydroperoxides, which are very unstable and further react to form secondary products such as hydrocarbons, alcohols, ketones and aldehydes, which can be oxidized to carboxylic acids. The quantitative determination of oxidation is very difficult. Classical methods of studying oxidation reactions refer to only one class of compounds present in the complex mixture formed during the oxidation process. Therefore, they offer only limited information about the oxidation process (Guillén & Ruiz, 2006). The peroxide value is useful in monitoring the initial stage of oxidation, because primary oxidation products are measured. However, the use of peroxide value as a measure of lipid oxidation is limited, because it decreases as oxidation proceeds due to rapid decomposition of hydroperoxides, which are very unstable at elevated temperature. The thiobarbituric acid test measures secondary products of oxidation of fatty acids with at least three double bonds and it is therefore not applicable to olive oil (van Loon, Linsen, Legger, & Voragen, 2006). The anisidine test is another possibility for assessing advanced oxidative rancidity (Labrinea, Thomaidis, & Georgiou, 2001).

The changes in fats and oils after heating or frying procedures have been the subject of numerous studies and experimental investigations. All chemical changes of fats and oils at elevated temperatures originate in oxidation, hydrolysis, polymerisation, isomerisation or cyclisation reactions (Quiles et al., 2002; Valavanidis et al., 2004). These reactions affect the sensorial, nutritional and safety properties of oil. All these reactions may be promoted by oxygen, moisture, traces of metal and free radicals (Quiles et al., 2002). Several factors, such as contact with air, temperature and length of heating, type of vessel, degree of oil unsaturation, and the presence of pro-oxidants or antioxidants, affect the overall performance of oil (Andrikopoulos, Kalogeropoulus, Falirea, & Barbagianni, 2002). In autoxidation, virgin olive oil stability has been correlated with the polar phenols content (Tsimidou, 1998). According to Blekas, Tsimidou, and Boskou (1995) the presence of biophenols protects oil in the initial stage of oxidation. When the activity of the polar phenolic fraction is reduced and primary products of autoxidation reach a critical concentration, α-tocopherol becomes effective. In the early stages of autoxidation, α-tocopherol can act as a pro-oxidant, depending on its concentration. α-tocopherol can also have synergistic effect with some phenolic compounds (Baldioli et al., 1996). The loss of antioxidants during heating depends on the surface/volume ratio (Andrikopoulos et al., 2002).

Consumption of the products of oxidized fat seems to be involved in several pathological conditions (Deiana et al., 2002). The choice of oil for use in frying is complex, as factors such as stability, price and nutritive value must be considered. The production of olive oil in Slovenia was 260 tons in 2004 and 937 tons were imported, mostly from Italy, Spain and Greece. Only virgin olive oils are produced in Slovenia, which are therefore more expensive than most imported olive oils. Research on virgin olive oil from Slovenian Istra, the major olive-growing area in Slovenia, began in the last decade of the 20th century, but no study on oxidative stability or study under accelerated conditions has been conducted so far. The aim of this study was to establish the chemical changes occurring in oil after exposure to high temperature and air for a period of time equalling or even exceeding the induction period determined with the Rancimat.

2. Materials and methods

2.1. Materials

Seven samples of extra virgin olive oil from Slovenian Istra were collected from local olive oil growers. Only good quality oils with known origin were included in the study. Four samples were of cv. *Istrska belica* and three of cv. *Leccino*. These are the two prevalent cultivars in Slovenian production. Cv. *Istrska belica* (Istrian white olive) is a domesticated variety (Bandelj Mavsar et al., 2005). It is grown in Slovenian Istra and the neighbouring countries. Cv. *Leccino* originates in Tuscany, Italy. All oil samples were produced in continuous extraction systems. The samples were stored in full dark glass bottles at 12 °C for five months before the experiments were performed.

2.2. Thermal treatment

Heat treatment of oil samples was conducted in a Rancimat 679 apparatus (Metrohm, Herisau, Switzerland). The samples were passed through anhydrous sodium sulphate before the experiment. The vessels were filled with 20 g of oil and the same operating conditions were set as for the determination of stability (100 °C, flow of air 10 L/h). After 142 h, when the conduction curve of the most stable sample started to increase rapidly, the experiment was stopped. The samples were transferred to glass vials under a nitrogen atmosphere and kept in the dark at −20 °C prior to analyses.

The analyses were performed on oils before heating and after heating. All analyses were performed in duplicate.

2.3. Quality parameters

Acidity was determined according to standard method (ISO 660., 1996) and was expressed as % of oleic acid. Peroxide value was determined according to standard method (ISO 3960., 2005) and was expressed as mmol O_2/kg. UV spectrophotometry (K_{232}, K_{270} and ΔK) was conducted following the analytical method described in the Regulation EEC 2568/91 (EEC, 1991).

2.4. Fatty acid composition

Determination of fatty acid composition was carried out according to the Regulation EEC 2568/91 (EEC, 1991). Methyl-esters were prepared with 2 M methanolic potassium hydroxide solution and analyzed by a GC equipped with a flame ionisation detector. A Hewlett-Packard gas chromatograph HP 6890 with an HP-INNOWax column (60 m length × 0.25 mm i.d., film thickness 0.25 μm) was used. The working conditions were as follows: carrier gas: nitrogen; carrier gas pressure at the beginning of the analysis: 307 kPa; split ratio: 100:1; temperature programme: 210 °C, 50 min, 15 °C/min, 240 °C, 5 min. The amount of an individual fatty acid is presented as a percentage of the total fatty acids.

2.5. Tocopherol content

The tocopherol content was determined by high-performance liquid chromatography (HPLC) according to the standard method (ISO 9936., 1997). Analyses were performed on an Agilent 1100 Series HPLC system chromatograph equipped with a BinPump G1312A binary pump, an ALS G1329A thermostatted autosampler, an ALSTherm G1330B autosampler thermostat, a COLCOM G1316A thermostatted column compartment, a Phenomenex Luna (250 × 4.60 mm) column packed with silica (5 μm particle size), an FLD G1321A fluorescence detector operating at an excitation wavelength of 290 nm and an emission wavelength of 330 nm. The mobile phase used was hexane–isopropanol (99.3:0.7 v/v) at a flow-rate of 1.0 mL/min. The isomers eluted in the order from α-tocopherol to δ-tocopherol, with the retention time of α-tocopherol being 6.6 min. Only α-tocopherol data are presented here. A five level calibration graph was constructed covering the concentration range from 30 to 1070 mg per kg of oil (mg/kg). The exact concentration of tocopherol stock standard, which served as the basis for five working calibration standards, was calculated from the measured absorbance of the standard and the known absorbance coefficient for the isomer. The calibration graph was used to evaluate the tocopherol content in the samples. The limit of quantification was determined and the expanded measurement uncertainty (U) with a coverage factor of 2, as well.

2.6. Total biophenol content

The total biophenol content of the oils was determined colorimetrically using the Folin–Ciocalteu reagent (Gutfinger, 1981). Five gram of oil was dissolved in 25 mL of hexane. Biophenols were extracted with three 10 mL portions of methanol/water (60/40, w/w). The combined extracts were evaporated to dryness using a rotary evaporator at 40 °C. The dry matter was dissolved in 1 mL of methanol. A 100 μL aliquot of the extract and 0.5 mL of Folin–Ciocalteu reagent were added to 5 mL of water in a 10 mL measuring flask. After 3 min 1 mL of a saturated solution of sodium carbonate was added and the flask was filled with water to the 10 mL mark. After 1 h, the extinction was measured at 725 nm. The amount of biophenols was calculated using a calibration curve prepared with caffeic acid. The results are expressed as mg of caffeic acid per kg of oil.

2.7. Biophenol composition

The extracts prepared for total biophenol content determination were also used for the determination of biophenol composition. An Agilent 1100 Series HPLC system chromatograph equipped with a BinPump G1312A binary pump was used together with a thermostatted ALS G1329A autosampler, an ALSTherm G1330B autosampler thermostat, a thermostatted COLCOM G1316A column compartment, and a multiwavelength detector. A Phenomenex LUNA ODS2 (5 μm i.d., 250 × 4.6 mm) column was used. The determinations were performed according to a modified method published by Cortesi, Rovellini, and Fusari (2002). The separation was achieved with an elution gradient using an initial composition of 96% mobile phase A (1000 mL of water and 2 mL of 98% phosphoric acid) and 4% mobile phase B (acetonitrile and methanol, 1:1 V/V). The concentration of mobile phase A was reduced to 50% at 40 min, 40% at 45 min and 0% at 60 min. 100% mobile phase B was maintained for the next 10 min and then it was reduced back to 4% in two minutes. In order to achieve better resolution, the column compartment temperature was kept at 15 °C. Detection was performed at 280 nm, with the exception of the flavonoids luteolin and apigenin, which were recorded at 340 nm. Calibration graphs for hydroxytyrosol, tyrosol, luteolin and apigenin were constructed using reference compounds. All the other biophenols were quantified using the response factor for tyrosol. Hydroxytyrosol was synthesized from 3,4-dihydroxyphenylacetic acid (Baraldi, Simoni, Manfredini, & Menziani, 1983).

2.8. Oil stability

To evaluate oil stability, the induction time for oxidation was measured by the Rancimat 679 apparatus (Metrohm, Herisau, Switzerland). The samples were passed through anhydrous sodium sulphate before the experiment. Three gram of oil were heated at 100 °C with a flow of air (10 L/h). The results are expressed as induction time (h).

2.9. Statistics

Statistical analysis was performed with SAS/STAT (SAS Software, Version 8.01, 1999). Basic statistical parameters were calculated with the MEANS procedure. The data were tested for normality of distribution with the UNIVARIATE procedure. The GLM (General Linear Model) was used for calculations. The statistical model included the influence of cultivar and of thermal

treatment and the interaction of both influences. The median values for experimental groups were calculated by the Duncan test and were compared at the 5% risk level.

Table 1
Quality parameters and oxidative stability of *Istrska belica* and *Leccino* extra virgin olive oils before and after thermal treatment for 142 h at 100 °C with a flow of air of 10 L/h

Parameter	Cultivar	Thermal treatment	
		Before $M \pm SD$	After $M \pm SD$
Acidity (% oleic acid)	*Istrska belica*	0.13 ± 0.01^{aB}	0.20 ± 0.03^{bA}
	Leccino	0.08 ± 0.01^{bB}	1.94 ± 1.30^{aA}
Peroxide value (mmol O_2/kg)	*Istrska belica*	3.6 ± 1.6^{bB}	30.1 ± 1.8^{bA}
	Leccino	6.0 ± 1.8^{aB}	359 ± 68^{aA}
K_{232}	*Istrska belica*	1.811 ± 0.057^{aB}	3.000 ± 0.089^{bA}
	Leccino	1.805 ± 0.117^{aB}	3.373 ± 0.126^{aA}
K_{270}	*Istrska belica*	0.155 ± 0.010^{aB}	0.518 ± 0.051^{bA}
	Leccino	0.141 ± 0.006^{bB}	1.503 ± 0.188^{aA}
ΔK	*Istrska belica*	-0.002 ± 0.001^{aB}	0.013 ± 0.002^{bA}
	Leccino	-0.002 ± 0.001^{aB}	0.020 ± 0.009^{aA}
Induction period (h)	*Istrska belica*	114.3 ± 9.9^{a}	–
	Leccino	78.4 ± 4.6^{b}	–

The data represent mean (M) and standard deviation (SD). The data reported are means of two independent replicate analyses for four *Istrska belica* or three *Leccino* samples of oils.
[a,b] Significant differences in the same column are shown by different small letters ($P < 0.05$).
[A,B] Significant differences in the same row are shown by different capital letters ($P < 0.05$).

Table 2
Fatty acid composition (given as % of total fatty acids) of *Istrska belica* and *Leccino* extra virgin olive oils before and after thermal treatment for 142 h at 100 °C with a flow of air of 10 L/h

Fatty acid	Cultivar	Thermal treatment	
		Before $M \pm SD$	After $M \pm SD$
C 14:0	*Istrska belica*	0.018 ± 0.046^{aA}	0.018 ± 0.005^{aA}
	Leccino	0.010 ± 0^{bB}	0.017 ± 0.005^{aA}
C 16:0	*Istrska belica*	12.759 ± 0.294^{bA}	12.733 ± 0.269^{bA}
	Leccino	13.805 ± 0.365^{aB}	16.430 ± 1.981^{aA}
C 16:1	*Istrska belica*	1.143 ± 0.098^{bA}	1.115 ± 0.086^{bA}
	Leccino	1.533 ± 0.811^{aA}	1.478 ± 0.062^{aA}
C 17:0	*Istrska belica*	0.060 ± 0^{aA}	0.058 ± 0.005^{bA}
	Leccino	0.050 ± 0^{bB}	0.063 ± 0.005^{aA}
C 17:1	*Istrska belica*	0.110 ± 0.009^{aA}	0.110 ± 0.009^{aA}
	Leccino	0.113 ± 0.005^{aA}	0.113 ± 0.008^{aA}
C 18:0	*Istrska belica*	$3.210 \pm .0118^{aA}$	3.278 ± 0.117^{aA}
	Leccino	2.283 ± 0.122^{bB}	2.823 ± 0.126^{bA}
C 18:1	*Istrska belica*	75.349 ± 0.795^{aA}	75.734 ± 0.768^{aA}
	Leccino	72.739 ± 0.364^{bA}	73.900 ± 1.985^{bA}
C 18:2	*Istrska belica*	5.739 ± 0.071^{bA}	5.373 ± 0.442^{aA}
	Leccino	7.965 ± 0.301^{aA}	2.437 ± 1.352^{bB}
C 18:3	*Istrska belica*	0.625 ± 0.033^{bB}	0.545 ± 0.026^{aA}
	Leccino	0.772 ± 0.31^{aA}	0.090 ± 0.077^{bB}
C 20:0	*Istrska belica*	0.481 ± 0.015^{aB}	0.504 ± 0.025^{aA}
	Leccino	0.335 ± 0.200^{bB}	0.445 ± 0.020^{bA}
C 20:1	*Istrska belica*	0.296 ± 0.015^{aA}	0.303 ± 0.019^{aA}
	Leccino	0.255 ± 0.012^{bB}	0.273 ± 0.014^{bA}
C 22:0	*Istrska belica*	0.145 ± 0.008^{aB}	0.169 ± 0.016^{bA}
	Leccino	0.097 ± 0.012^{bB}	1.867 ± 1.231^{aA}
C 24:0	*Istrska belica*	0.059 ± 0.010^{aA}	0.063 ± 0.005^{aA}
	Leccino	0.033 ± 0.005^{bA}	0.073 ± 0.044^{aA}

The data represent mean (M) and standard deviation (SD). The data reported are means of two independent replicate analyses for four *Istrska belica* or three *Leccino* samples of oils.
[a,b] Significant differences in the same column are shown by different small letters ($P < 0.05$).
[A,B] Significant differences in the same row are shown by different capital letters ($P < 0.05$).

3. Results and discussion

This investigation included good quality samples of extra virgin olive oils from cv. *Istrska belica* and cv. *Leccino*, which are the predominant cultivars in Slovenian Istra.

The values of physical and chemical parameters in oils before and after thermal treatment are given in Tables 1–3. As expected, most of the measured physico-chemical parameters changed during heating. The changes were greater in *Leccino* samples, which all had shorter induction periods measured with the Rancimat apparatus than the *Istrska belica* samples. The heating treatment lasted 142 h, until the induction period (in 20 g of sample) of the three most stable olive oil samples was determined. Three *Istrska belica* samples had approximately the same induction period, the fourth *Istrska belica* sample was heated 19 h beyond its induction time and the *Leccin*o samples were heated 26–38 h beyond their induction time.

3.1. Quality parameters

The increase in acidity during heating of fats and oils can be caused by the hydrolysis of triacylglycerols as well as by the formation of secondary oxidation products, namely volatile carboxylic acids, such as formic or acetic acid. In our study, the acidity increase was larger in *Leccino* olive oils than in *Istrska belica* olive oils.

The most evident difference between the two cultivars was the change of *peroxide value*. After heating, the peroxide values of *Leccino* samples were all over 300 mmol O_2/kg, while in *Istrska belica* the highest measured peroxide value after heating was 32.4 mmol O_2/kg. In a similar study, Nissiotis and Tasioula-Margari (2002) heated oil samples for 100 h at 100 °C in an oven. The peroxide numbers increased to 71.25–105.91 meq/kg (35.63–52.96 mmol O_2/kg), which are similar to the results for *Istrska belica* olive oils in our study. The peroxide values in our study increased although the biophenol content was still relatively high. Antioxidants in oil react with radicals, and the peroxide value is expected to increase only when insufficient antioxidants are left to compensate for radical formation. On the other hand, the formation of peroxides is a chain reaction that occurs so rapidly that peroxide formation and radical scavenging start, apparently, simultaneously (van Loon et al., 2006).

Table 3
α-tocopherol and biophenols contents (in mg/kg) of *Istrska belica* and *Leccino* extra virgin olive oil before and after thermal treatment for 142 h at 100 °C with a flow of air of 10 L/h

Compound	Cultivar	Thermal treatment	
		Before $M \pm SD$	After $M \pm SD$
α-tocopherol	*Istrska belica*	129 ± 17^{b}	<2
	Leccino	390 ± 169^{a}	<2
Total biophenols	*Istrska belica*	598 ± 74^{aA}	240 ± 47^{aB}
	Leccino	399 ± 40^{bA}	176 ± 18^{bB}
Luteolin	*Istrska belica*	3.1 ± 0.6^{aA}	1.8 ± 0.4^{aB}
	Leccino	1.8 ± 0.2^{bA}	0.1 ± 0.1^{bB}
Apigenin	*Istrska belica*	1.8 ± 0.5^{aA}	1.6 ± 0.4^{aA}
	Leccino	0.7 ± 0.2^{bA}	0.4 ± 0.2^{bB}
Lignans	*Istrska belica*	38.3 ± 7.6^{aB}	101.0 ± 26.5^{aA}
	Leccino	10.0 ± 2.1^{bA}	20.3 ± 11.9^{bA}
Ole	*Istrska belica*	17.3 ± 2.2^{aA}	0^{aB}
	Leccino	6.4 ± 1.8^{bA}	0^{aB}
O-Agl	*Istrska belica*	83.1 ± 20.8^{aA}	5.7 ± 1.7^{aB}
	Leccino	27.8 ± 5.0^{bA}	1.3 ± 0.3^{bB}
L-Agl	*Istrska belica*	46.3 ± 20.6^{aA}	12.1 ± 2.2^{aB}
	Leccino	9.6 ± 3.5^{bA}	6.0 ± 2.5^{bA}
O-Agl-dA	*Istrska belica*	23.8 ± 2.7^{aA}	2.0 ± 1.2^{aB}
	Leccino	5.3 ± 2.4^{bA}	1.5 ± 0.6^{aB}
L-Agl-dA	*Istrska belica*	31.3 ± 5.0^{aA}	1.6 ± 1.4^{aB}
	Leccino	3.5 ± 1.5^{bA}	1.9 ± 1.4^{aA}
DMO-dA	*Istrska belica*	165.5 ± 35.0^{aA}	4.6 ± 1.4^{aB}
	Leccino	184.2 ± 21.9^{aA}	4.7 ± 2.0^{aB}
DML-dA	*Istrska belica*	182.0 ± 32.8^{aA}	20.9 ± 4.7^{aB}
	Leccino	132.3 ± 25.5^{bA}	6.8 ± 3.5^{bB}
TyrOH	*Istrska belica*	8.0 ± 6.2^{aA}	3.6 ± 2.8^{aA}
	Leccino	2.5 ± 0.4^{aA}	0^{bB}
Tyr	*Istrska belica*	11.0 ± 8.7^{aB}	18.4 ± 3.3^{aA}
	Leccino	3.0 ± 0.3^{bB}	13.9 ± 2.7^{bA}

The data represent mean (M) and standard deviation (SD). The data reported are means of two independent replicate analyses for four *Istrska belica* or three *Leccino* samples of oils.
[a,b] Significant differences in the same column are shown by different small letters ($P < 0.05$).
[A,B] Significant differences in the same row are shown by different capital letters ($P < 0.05$).

K_{232} and K_{270} are simple and useful parameters for assessing the state of oxidation of olive oil. K_{232} is a measure of the primary oxidation products, conjugated dienes, which are formed by a shift in one of the double bonds. K_{270} is increased by conjugated trienes (the primary oxidation products of linolenic acid) and secondary products of oxidation, such as aldehydes and ketones (Koprivnjak, 2006). Both spectrophotometric parameters increased during heating. The increase of K_{270} was much greater than the increase of K_{232}, probably because most of the primary oxidation products underwent further oxidation. Oxidation was more extensive in *Leccino* oils because these samples were heated for several hours after they reached their induction time.

3.2. Fatty acid composition

The fatty acid composition of olive oils before and after thermal treatment is given in Table 2. There is only a slight difference between the fatty acid compositions of the fresh samples of the two cultivars.

The changes in the fatty acid composition in *Istrska belica* oils and *Leccino* oils are presented in Fig. 1. The percentages of fatty acids below the dashed line decreased during heating, and the percentages of fatty acids above the dashed line increased during heating. The larger the distance of the data point from the dashed line, the greater is the change in content of fatty acid.

The changes of fatty acid composition give us an insight into the kinetics of fatty acid oxidation reactions. It is evident from Fig. 1b that the linolenic acid content was reduced the most, followed by linoleic acid. After heating, there was a significant increase in the contents of saturated fatty acids, while the contents of monounsaturated acids, including the predominating fatty acid oleic acid, remained unaltered. These observations are in agreement with an $_1$H nuclear magnetic resonance study, which confirmed the fact that the fatty acid degradation rate increases with the number of double bonds in the molecule (Guillén & Ruiz, 2006).

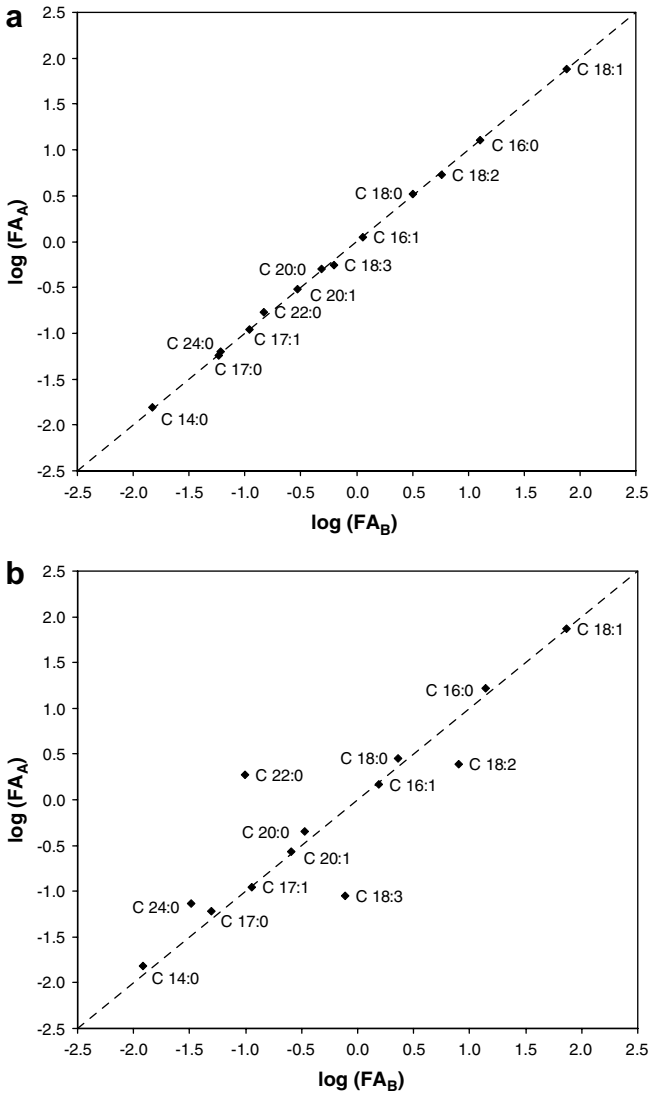

Fig. 1. Relationship between the content of individual fatty acids before and after thermal treatment (FA_B – % of fatty acid before thermal treatment; FA_A – % of fatty acid after 142 h at 100 °C with a flow of air of 10 L/h). a: cv. *Istrska belica*; b: cv. *Leccino*.

3.3. α-tocopherol

The content of α-tocopherol was higher in *Leccino* olive oils than in *Istrska belica* oils, which is in agreement with previous research (Butinar, Bučar-Miklavčič, & Čalija, 1999a). After heating α-tocopherol was depleted in all samples, so that the contents were then below the limit of quantification (2 mg/kg). Nissiotis and Tasioula-Margari (2002) also reported that α-tocopherol was completely depleted during 100 h heating at 100 °C, even when the oxidation was not accelerated by bubbling air through the sample.

3.4. Biophenols

Total biophenol content in *Istrska belica* olive oils is higher than in *Leccino* olive oils (Butinar, Bučar-Miklavčič, & Čalija, 1999b), which is in accordance with the results given in Table 3. The total biophenol content measured with Folin–Ciocalteu reagent was not completely depleted after heating; it decreased by 60% in *Istrska belica* and by 55% in *Leccino*. The total biophenol content is not sufficient to provide a good insight into the protection of an oil against oxidation, so we should consider the changes in the amounts of individual biophenols. Four groups of biophenols are found in virgin olive oils; namely simple biophenols, lignans, flavonoids and the predominating group of secoiridoid biophenols. Only complex biophenols and flavonoids are reported here. The three lignans from olive oil coelute in a single peak (Fig. 2a) and therefore the sum of all three compounds is given.

Oleuropein and ligstroside are characteristic secoiridoid biophenols from the olive drupe. They can enter different

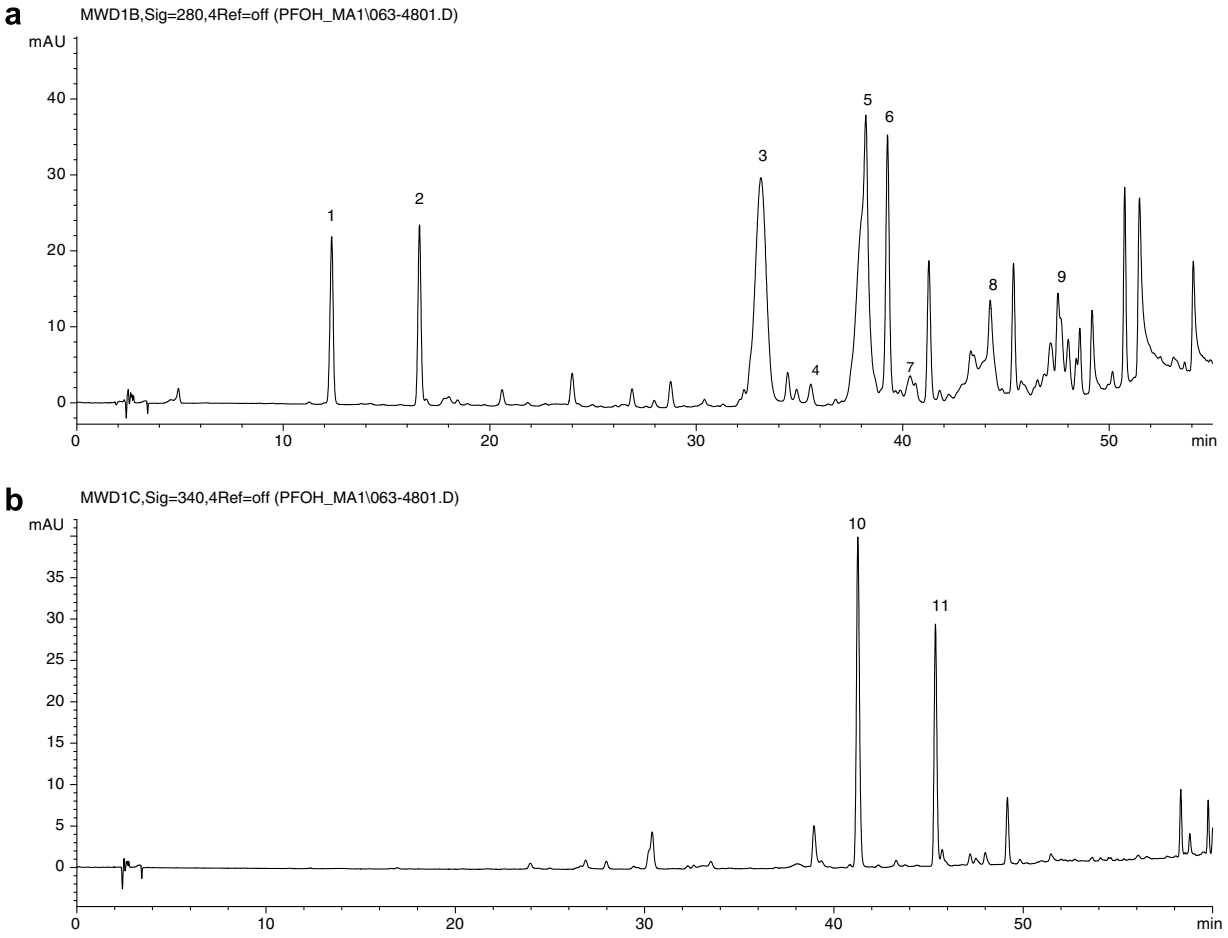

Fig. 2. HPLC chromatograms of biophenols determination (a: 280 nm; b: 340 nm). Peaks correspond to (1) hydroxytyrosol, (2) tyrosol, (3) decarboxymethyl oleuropein aglycon, (4) dialdehydic form of oleuropein aglycon, (5) dialdehydic form of demethoxyligstroside aglycon, (6) lignans, (7) dialdehydic form of ligstroside aglycon, (8) oleuropein aglycon, (9) ligstroside aglycon, (10) luteolin and (11) apigenin.

transformation-reaction pathways, which start in an injured drupe or during oil extraction and continue in the oil during its whole life cycle. Oleuropein is first transformed to the (closed) aldehydic or hydroxy form of oleuropein aglycon (O-Agl), which is further converted to the dialdehydic form of oleuropein aglycon (O-Agl-dA), then to the prevailing dialdehydic form of decarboxymethyl oleuropein aglycon (DMO-dA), and finally to the aromatic alcohol hydroxytyrosol (TyrOH). An identical transformation occurs for ligstroside: first from ligstroside to the ligstroside aglycon (L-Agl), from L-Agl to the dialdehydic form of ligstroside aglycon (L-Agl-dA), further to the decarboxymethyl ligstroside aglycon (DML-dA) and finally to the aromatic alcohol tyrosol (Tyr). As long as secoiridoids are not transformed to their final forms TyrOH and Tyr, the oils can preserve their freshness, fruitiness and harmony. When the transformation pathway approaches its end, the total biophenol content (determined spectrophotometrically using the Folin–Ciocalteu reagent) can still be relatively high. Nevertheless, the oil has already lost its freshness, and is poorly shielded against autoxidative decay (Bandelj Mavsar et al., 2005). This is due to the high amount of tyrosol, which is not as strong an antioxidant as hydroxytyrosol and the secoiridoids with a hydroxytyrosol moiety. The rate of hydroxytyrosol degradation during heating olive oil is higher than that of tyrosol, confirming the higher antioxidant power of the former (Cheikhousman et al., 2005).

The *biophenol compositions* of olive oil before and after thermal treatment are given in Table 3. The data show that secoiridoid biophenols prevailed in all samples before thermal treatment. The average amount of each biophenol was higher in *Istrska belica* samples than in *Leccino* samples, with DMO-dA being the only exception. It is evident from Fig. 3a that the transformation of biophenols in *Leccino* oils was more advanced than in *Istrska belica*.

The amounts of biophenols in thermally treated olive oils are given in Table 3 and Fig. 3b. The depletion of both flavonoids was greater in *Leccino* olive oils than in *Istrska belica* oils. The amounts of lignans, on the contrary, increased during heating, except in one sample of *Leccino*. Brenes, García, Dobarganes, Velasco, and Romero (2002) reported that lignans are stable compounds but this statement does not explain the increase of lignans during

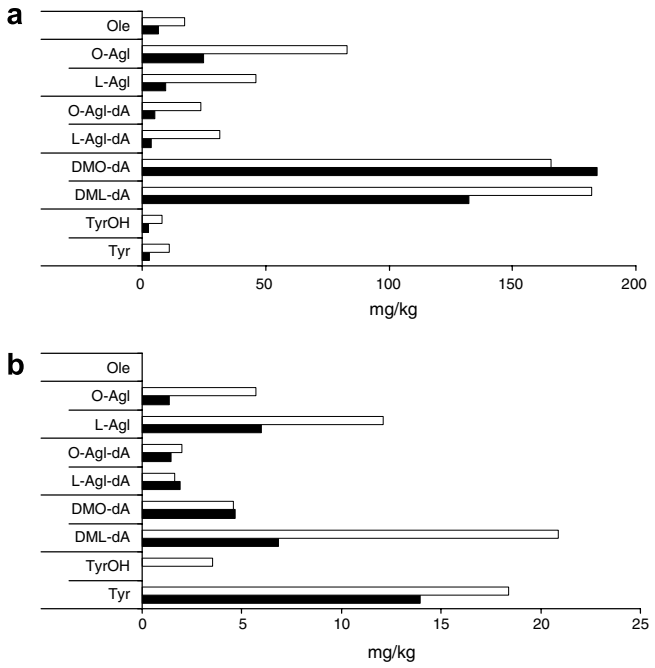

Fig. 3. Amounts of secoiridoid biophenols in cv. *Istrska belica* olive oils (□) and cv. *Leccino* olive oils (■). a: before thermal treatment; b: after 142 h at 100 °C with a flow of air of 10 L/h. (Ole – oleuropein, O-Agl – oleuropein aglycon, L-Agl – ligstroside aglycon, O-Agl-dA – dialdehydic form of oleuropein aglycon, L-Agl-dA – dialdehydic form of ligstroside aglycon, DMO-dA – dialdehydic form of demethoxyoleuropein aglycon, DML-dA – dialdehydic form of demethoxyligstroside aglycon, TyrOH – hydroxytyrosol, Tyr – tyrosol). Note the different scale of the two graphs.

heating. It is possible that some oxidized compound coelutes with lignans, causing an increase of the peak identified as the lignan peak, as is the case with L-Agl-dA (Brenes et al., 2002; Rovellini & Cortesi, 2002).

The amounts of secoiridoid biophenols decreased significantly. There were only traces of hydroxytyrosol in oils after heating. The amount of tyrosol, the final phenol derivative of ligstroside transformations, increased significantly, especially in *Istrska belica* samples. In a similar study, Nissiotis and Tasioula-Margari (2002) used an oil sample with much higher initial levels of hydroxytyrosol and tyrosol (namely 80.96 and 105.42 mg/kg). The level of hydroxytyrosol was reduced to 2.75 mg/kg, but the level of tyrosol remained much higher at 57.29 mg/kg. The results are in agreement with the tests on the antioxidative properties of the studied compounds; the antioxidant capacity decreased in the order TyrOH > O-Agl > L-Agl > Tyr (Carrasco-Pancorbo et al., 2005).

3.5. Oxidative stability

Istrska belica olive oils had longer induction periods than *Leccino* olive oils. This is probably due to the fatty acid composition and the content of antioxidants. We found a strong negative correlation between the amount of polyunsaturated fatty acids and the oxidative stability, while a statistically less significant positive correlation was found between the amount of monounsaturated fatty acids and the oxidative stability. The amount of saturated fatty acids did not correlate with the oxidative stability.

We found no linear correlation between the spectrophotometric data and oxidative stability while there was a negative correlation between the peroxide value and the oxidative stability.

Phenolic compounds seem to be more effective than α-tocopherol in protecting olive oil from oxidation (Rastrelli, Passi, Ippolito, Vacca, & De Simone, 2002). Baldioli et al. (1996) showed that the oxidative stability of virgin olive oils correlated mainly with the content of biophenols and in particular with secoiridoids containing the hydroxytyrosol moiety. Synergistic and antagonistic effects between different components of olive oil are known (Mateos, Trujillo, Perez-Camino, Moreda, & Cert, 2005). In our study, strong correlations were found between oxidative stability and the amounts of individual biophenols, the strongest correlations being those of O-Agl-dA, L-Agl-dA and oleuropein. The correlation between α-tocopherol and oxidative stability was negative but statistically less significant. This observation might mislead us to the conclusion that α-tocopherol has a negative influence on the stability of olive oil, but a detailed look at the composition of the two kinds of samples offered another possible explanation. In the *Istrska belica* samples, which contain less α-tocopherol than the *Leccino* samples, the amounts of biophenols were higher and the unsaturation of the fatty acids was lower. Even more, the biophenol composition showed that this pool of antioxidants was already partly depleted in untreated *Leccino* olive oils. It seems that the influence of biophenols and fatty acid composition on oil stability was stronger than the influence of α-tocopherol. Oxidative stability is therefore not dependent on a single parameter, but is rather affected by fatty acid composition and a complex pool of antioxidants and pro-oxidants.

4. Conclusion

Extra virgin olive oils from two Slovenian cultivars were evaluated for the first time to identify their oxidative stability and the influence of heating on physico-chemical parameters. *Istrska belica* extra virgin olive oils had longer induction periods than *Leccino oils*. There were more oxidation products and more intense changes in fatty acid composition in *Leccino* oils after 142 h of heating to 100 °C. α-tocopherol was completely depleted in all samples, while secoiridoid biophenols underwent transformations towards their simple phenol analogues tyrosol and hydroxytyrosol. However, the experimental conditions in the study presented were very severe (a long heating time and air bubbling) and all the parameters were only measured at the beginning and at the end of heating. Hence, further investigations will be necessary in order to draw conclusions about how long oil can be heated before the deterioration increases to such a level that it is no longer acceptable for human consumption.

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