Influence of Small Amount of Water Addition in the Extraction Process on the Olive Oil Yield and Phenolic Compounds

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Summary

Climate changes have a significant influence on rainfall quantity, on the loss of soil humidity and consequently loss of water in olive fruit. The loss of fruit moisture could result in dry olive paste during oil production, which makes extraction of olive oil difficult and could lead to lower oil yield. Addition of water during the malaxation process is suggested to overcome this problem, but this could cause a reduction of the phenolic compounds in obtained oil. The aim of this study was to investigate the effect of the addition of a small amount of water (5% w/w) in 'Leccino' cv. olive paste during malaxation on the oil yield, extractability index and oil phenolic profile. The intention was to investigate whether it was possible to achieve the benefit of improved oil extractability and at the same time preserve the major proportion of valuable phenolic compounds. The addition of 5% of water to the olive paste during malaxation had no influence on oil yield and extractability index. On the other hand, water addition caused an increase in the concentration of total secoiridoids and total flavonoids, while total simple phenolic compounds decreased. The obtained results pointed out that even if a small amount of water addition during malaxation had no significant influence on oil yield and extractability index, the resulting changes in oil phenolic profile indicated the possibility of a positive influence on its oxidative stability, and consequently extension of its shelf life.

Key words

virgin olive oil, water addition, malaxation, oil yield, phenolic compounds

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Introduction

Virgin olive oil (VOO) is widely consumed in the Mediterranean area as a basic part of the Mediterranean diet due to its beneficial nutritional characteristics, mainly related to its favourable fatty acid composition and phenolic content and profile (Lazzez et al., 2008; Bendini et al., 2007). Olive oil characteristics and composition depend on the characteristics of the olive fruits (cultivar, sanitary condition, etc.), as well as on the environmental and processing conditions in olive oil production (Di Giovacchino et al., 2002; Brkić Bubola et al., 2012; Lukić et al., 2017). Production of high-quality VOO at the highest yield and minimum cost, using environmentally friendly processes in the olive oil production, is more and more demanded (García González and Aparicio, 2010). Production of VOO is a mechanical process that consists of crushing, malaxation and oil separation, where malaxation is the most important step including several enzymatic processes in the olive pastes (Clodoveo et al., 2014) that significantly define the final oil aromatic and phenolic profile.

The Mediterranean area, where more than 90% of the world's olive oil is produced, is affected by the climate change, which may negatively affect growth and productivity of olive plants (Dell'Aquila et al., 2012; Gualdi et al., 2013; Dag et al., 2014). Climate changes, such as higher temperature and waterfall deficit cause problems in olive cultivation and oil production, because they directly affect the quantity and quality of produced olive oil (Tura et al., 2008; Ozdemir, 2016). Water deficit affects the loss of soil humidity and consequently the loss of water in olive fruit. The loss of moisture in the fruits results in a dry olive paste during oil production, which makes extraction of olive oil difficult and could cause a lower oil yield. In addition to other parameters, the water content of the olive paste is a dominant factor that determines the efficiency of the extraction process and other oil quality parameters (Ben-David et al., 2010). To improve the oil extractability, the addition of water to the olive paste during malaxation was suggested, while the different effect on the quantity and quality of the oils obtained from different cultivars was also determined (Ben-David et al., 2010; Carrapiso et al., 2013). Most of the published literature referred to the influence of quite a high amount of water addition (25% - 40% w/w) during malaxation step on the total phenolic content in obtained oil (Ben-David et al., 2010; Carrapiso et al., 2013; Kiritsakis et al., 2017), which is considered to be the main cause of the reduction of the phenolic compounds content that could negatively influence the oxidative stability and the quality of the final product considering their direct interdependence. According to our knowledge, only a single study studied the addition of water (25% w/w) on the phenolic profile of olive oil (Kiritsakis et al., 2017), reporting also the negative influence on it. Therefore, many researchers have pointed out the importance of reducing the amount of water added (Di Giovacchino et al., 2001; Altieri et al., 2013). Due to proven negative effect of high amount of water addition during the olive oil production on their phenolic composition, currently in the olive oil industry only a small amount of water (5-10% w/w) is applied in order to improve extractability of olive paste. Therefore, the aim of this study was to investigate whether it is possible to achieve the benefit of improved oil extractability and at the same time preserve the major proportion of valuable phenolic compounds by adding a lower amount of water during malaxation, which has not been, to our knowledge, investigated to date. The specific aim was to investigate the effect of the addition of water (5% w/w) to 'Leccino' cv. olive paste during laboratory scale oil production on production parameters (oil yield and extractability index) and phenolic profile of the obtained oil.

Materials and Methods

VOO Sample Preparation

Olive (*Olea europeae* L.) fruits cv. 'Leccino' were harvested in October 2018 from the non-irrigated experimental field of the Institute of Agriculture and Tourism in Poreč, Croatia. Ripening index of the olive fruits determined according to Beltrán et al. (2004) was 1.95. Olive fruits were divided in six batches of 1 kg in order to process each batch separately into olive oil samples using an Abencor laboratory oil mill (MC2 Ingeneria y Sistemas, Sevilla, Spain). Three batches of fruits were processed into oil without the addition of water (control oil, L-0) and three batches (L-W) were processed with the addition of 5% w/w of water (which represented 5% of olive fruits from one batch) during malaxation process. For all the samples, fruits were crushed by a hammer mill and olive paste was malaxed for 30 minutes at 25 ± 1°C. Olive paste was centrifuged for 1 minute at 3500 rpm and the extracted oil was decanted. The olive oil samples (three L-0 and three L-W) were bottled in dark glass bottles and stored at 4 °C until analysis, which was conducted in two weeks after oil production.

Oil Yield, Moisture Content and Extractability Index

The oil yield (%) was calculated from three parallel processing repetitions, multiplying by 100 the mass ratio of mechanically extracted oil (g) and centrifuged olive paste (g) (Koprivnjak et al., 2016).

Olive paste samples (50 g), used for determination of the moisture and oil content, were divided from each batch during the malaxation process. Obtained olive paste samples were dried at 80 °C until constant weight, in order to determine the moisture content.

The fruit oil content on fresh mass (F) was determined according to the method described by Brkić et al. (2006) using Soxtec Avanti 2055 apparatus (Foss Tecator, Hilleroed, Sweden). Olive oil extractability index (EI) was calculated according to Beltrán et al. (2003) using the formula: $EI = V \times d / W \times F \times 100$, where V (mL) is the volume of extracted olive oil, d is the average olive oil density (0.915 g mL^{-1}), W (g) is the olive paste weight, and F (%) is the fruit oil content (on fresh mass) measured by Soxtec apparatus (Brkić et al., 2006).

Extraction and Determination of Phenolic Compounds

Phenolic compounds were extracted from olive oil samples following the method based on ultrasound assisted liquid-liquid extraction with methanol according to Jerman Klen et al. (2015) with some modifications described in Lukić et al. (2017). Chromatographic separation of phenolic compounds was performed on an HPLC-DAD system Agilent Infinity 1260 (Agilent Technologies, Santa Clara CA, USA) equipped with a G1311B quaternary pump, a G1329B autosampler, a G1316A

column oven, and a G4212B DAD detector. A Kinetex PFP column (2.6 µm, 100 mm x 4.6 mm) with a guard was used at 27 °C (Phenomenex, Sydney, Australia). The detection was carried out at 280 nm for simple phenolic compounds, lignans, secoiridoids, and vanillic acid, at 320 nm for vanillin and *p*-coumaric acid, and at 365 nm for flavonoids. The identification was performed by comparing retention times and UV/Vis spectra with those of pure standards when available, and with UV/Vis spectra from the literature (Jerman Klen et al., 2015). For quantification, standard calibration curves were made for tyrosol, hydroxytyrosol, vanillic acid, vanillin, *p*-coumaric acid, luteolin, apigenin, pinoresinol, and oleuropein. Based on the constructed calibration curves, concentrations of particular phenolic compounds and sums were expressed in mg kg^{-1} oil. Semiquantitative analysis was performed for hydroxytyrosol acetate, acetoxypinoresinol, and secoiridoids, where the concentrations were expressed as equivalent to those of hydroxytyrosol, pinoresinol, and oleuropein, respectively, assuming a response factor equal to one. The total phenolic content was presented as the sum of all the identified phenolic compounds. The analysis was conducted in three replicates per sample and mean values were used for further data elaboration.

Statistical Analysis

The effect of water addition on the investigated parameters was tested using one-way ANOVA at a 5% significance level, and mean values were compared using Tukey's honest significance difference test ($p \leq 0.05$). Statistical data elaboration was performed using Statistica v. 13.2 software (StatSoft. Inc., Tulsa, OK, USA).

Results and Discussion

Influence of Water Addition during Malaxation on Oil Yield and Extractability Index

Several studies have shown that the addition of water to olive paste during malaxation could increase the oil yield, since water as a co-adjuvant may help to break down water–oil emulsions, which makes oil extraction easier (Velasco and Dobarganes, 2002; Clodoveo, 2012). On the other hand, particular studies have reported a decrease in oil yield and EI as a consequence of water addition during malaxation (Valdivia et al., 2008; Ben-David et al., 2010). Rigane et al. (2020) have found that the effect of water addition during malaxation is cultivar dependant, probably due to different compositional characteristics of olive fruits, such as average water content, percentage of stone in the fruit, and flesh consistency (Ben-David et al., 2010). Our results showed that 5% w/w of water addition had no significant influence on oil yield (Figure 1a) nor on EI of 'Leccino' cultivar (Figure 1b).

Several factors could be the reason for that, but one of the most relevant could be the considerable water content in the 'Leccino' cv. olive paste (49%). The addition of water as a co-adjuvant for olive oil extraction is more suitable for low-moisture fruits (Valdivia et al., 2008), than for fruits with medium or high moisture content (50 - 70%), as it was in the case of 'Barnea' and 'Picual' cv. (Ben-David et al., 2010) as well as 'Hojiblanca' (Valdivia et al., 2008), where the water addition caused a decrease in oil yield. Because of that, it has been suggested to avoid the addition of water to the paste of fruits from irrigated orchards as they already contain considerable water levels (Ben-David et al., 2010). Carrapiso et al. (2013) also suggested that, in terms of oil yield, water addition should be avoided even with fruits from non-irrigated orchards, at least when they contained a moisture content as high as 53–56%. In that case, the addition of water at 42.9% w/w to olive paste during malaxation caused a decrease in oil yield and EI. A considerably lower amount of water added (5% w/w) in our study than in other studies (Ben-David et al., 2010; Carrapiso et al., 2013; Rigane et al., 2020) was probably among the main reasons for a non-significant influence on the oil yield and EI of 'Leccino' cultivar.

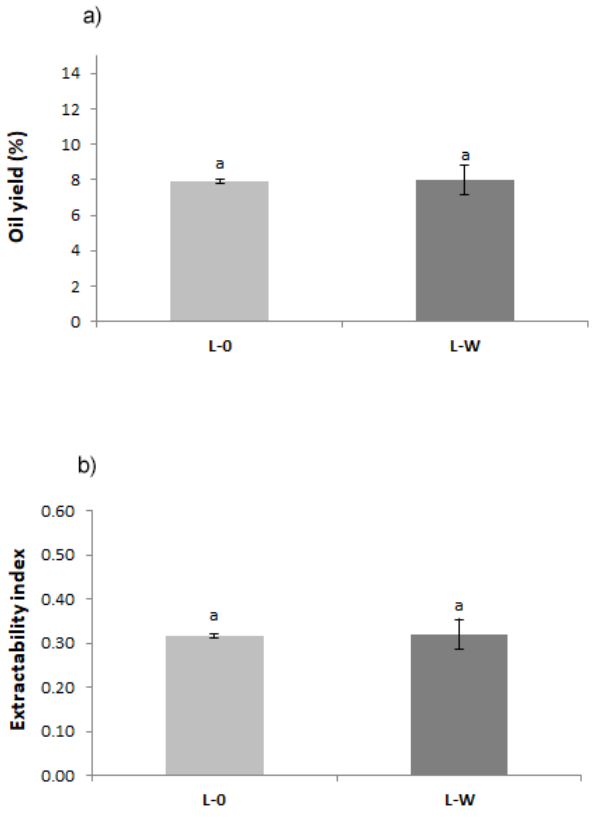

Figure 1. a) Oil yield (%) and b) extractability index determined in the production of 'Leccino' cv. olive oil obtained without (L-0) and with 5% w/w of water addition (L-W). Results are represented as mean values of three technical repetitions ± SD, bars labelled by different letters are significantly different (Tukey´s test, $P \leq 0.05$)

Influence of Water Addition on Phenolic Compounds in VOOs

In our study the addition of 5% w/w of water in 'Leccino' cv. olive paste had no influence on the total identified phenolic content (Table 1). Otherwise, Ben David et al. (2010) have reported different influence of water addition during malaxation between 'Picual' and 'Barnea' cv. olive pastes. In the case of 'Picual' cv. the addition of water at the rate between 0 and 300 cm3 (0 - 42% w/w) had no influence on the total phenolic compounds in the obtained oil, while higher rates led to a significant decrease in total phenolic content in 'Barnea' cv. oils. Carrapiso et al. (2013) also reported that the water addition at 43% (w/w) caused a decrease of the total phenolic content in 'Carrasqueña' and 'Picual' oils.

Table 1. Concentrations (mg kg^{-1}) of phenolic compounds in 'Leccino' cv. virgin olive oil obtained without (L-0) and with 5% of water addition (L-W) during malaxation

	L-0	L-W	Significance[a]
Simple phenolic compounds			
Tyrosol	13.25 ± 1.03	6.53 ± 0.41	***
Hydroxytyrosol	3.68 ± 0.21	3.92 ± 0.29	ns
Hydroxytyrosol acetate	2.37 ± 0.20	2.11 ± 0.23	ns
Vanillin	0.58 ± 0.07	0.53 ± 0.05	ns
Total simple phenolic compounds	19.88 ± 0.69	13.09 ± 0.85	***
Secoiridoids			
Elenolic acid glucoside (isomer)	1.78 ± 0.42	1.43 ± 0.09	ns
3,4-DHPEA-EDA	141.85 ± 38.22	222.33 ± 19.80	*
Oleuropein aglycone (isomer I)	47.88 ± 7.08	53.18 ± 4.28	ns
p-HPEA-EDA	95.00 ± 9.59	98.79 ± 0.78	ns
Oleuropein + ligstroside aglycones I & II	29.58 ± 1.51	25.05 ± 1.17	*
Oleuropein aglycone (isomer II)	4.90 ± 0.69	3.39 ± 0.29	*
Ligstroside aglycon (isomer III)	13.16 ± 0.90	16.52 ± 2.40	ns
Oleuropein aglycone (isomer III)	1.71 ± 0.16	1.13 ± 0.33	ns
Total secoiridoids	335.83 ± 39.15	421.82 ± 19.84	*
Flavonoids			
Luteolin	0.94 ± 0.11	1.21 ± 0.03	*
Apigenin	0.31 ± 0.02	0.32 ± 0.02	ns
Total flavonoids	1.25 ± 0.10	1.52 ± 0.01	*
Lignans			
Pinoresinol	4.27 ± 0.43	5.42 ± 0.67	ns
Acetoxypinoresinol	15.26 ± 1.33	12.94 ± 1.15	ns
Total lignans	19.52 ± 1.00	18.36 ± 1.82	ns
Phenolic acids			
Vanillic acid	1.48 ± 0.14	1.26 ± 0.19	ns
p-coumaric acid	0.85 ± 0.17	0.76 ± 0.14	ns
Total phenolic acids	2.33 ± 0.31	2.02 ± 0.30	ns
Total phenolic content (mg/kg)	376.48 ± 37.29	415.75 ± 68.98	ns

Results represent mean values of three repetitions ± SD. [a]Data were analysed by one-way ANOVA (ns, not significant; *, P ≤ 0.05; ***, P ≤ 0.001) and when differences were significant, mean separation was performed with Tukey's test (P ≤ 0.05)

The addition of water to the crushed olive fruits during three-phase processing, has been reported to be negatively related to the content of the total phenolic compounds (Cert et al., 1996; Ben-David et al., 2010), when compared to the two-phase decanter with a low water requirement (Di Giovacchino et al., 2001). The decrease in phenol content due to the water addition could be explained by the hydrophilic character of phenolic compounds (Spugnoli et al., 1999). The phenolic compounds are much less soluble in oil than in water and therefore, by limiting the amount of water added during oil extraction, better recovery of phenolic compounds in the oil could be achieved (Kalogeropoulos et al., 2014). In our case, the level of the added water was quite low (5% w/w) compared to the mentioned investigations (Ben-David et al., 2010; Carrapiso et al., 2013), which probably better preserved the amount of total phenolic compounds in oils obtained in this experiment.

The phenolic compounds of VOO belong to different classes: simple phenolic compounds, secoiridoids, flavonoids, lignans and phenolic acids, which have antioxidant properties and influence the oxidative stability of oils (Bendini et al., 2007). The water addition decreased the concentration of tyrosol by 51%, and consequently decreased the concentration of total simple phenolic compounds by 34% regarding control oil (Table 1). A reduction of simple phenolic compounds was also reported in the case of three-phase system where a large amount of warm water was used in the extraction process (Di Giovacchino et al., 2001), mainly due to their better solubility in water than in oily phases, which makes the amount of added water a crucial determinant for the concentration of phenolic compounds in the final product (Clodoveo and Hbaieb, 2013). Another simple phenol, hydroxytyrosol, was not changed by water addition, probably due to its good solubility in both oily and in aqueous media (Bouzid et al., 2005). This finding for hydroxytyrosol is in agreement with the results of Kiritsakis et al. (2017) obtained after addition of 25% (w/w) of water in 'Koroneiki' olive paste during malaxation process. The shelf life of VOO is highly influenced by the presence of phenolic molecules with catechol group, such as hydroxytyrosol and its corresponding secoiridoid derivatives (Bendini et al., 2007). Secoiridoids (aglycon forms) are released from their precursors in olive fruit paste after hydrolysis by endogenous β-glucosidases during crushing and malaxation. These aglycons have amphiphilic characteristics, and are partitioned between the oily layer and the vegetation water, but are more concentrated in water because of their polar functional groups (Bendini et al., 2007). In this work, the addition of 5% (w/w) of water in 'Leccino' olive paste during malaxation caused an increase in the concentration of total identified secoiridoids by 26% regarding control oil (Table 1), mainly due to an increase in the concentration of 3,4-DHPEA-EDA (dialdehyde of decarboxymethyl oleuropein aglycone), the most abundant secoiridoid in the oil. An increased concentration of 3,4-DHPEA-EDA could be explained by the degradative pathways of the phenolic oleosides by the enzymes during the malaxation process (De Leonardis et al., 2013) probably accelerated by water addition. Contrary to our results, Kiritsakis et al. (2017) have found a decrease in 3,4-DHPEA-EDA concentration in 'Koroneiki' oils obtained with the addition of water in malaxation at 25% (w/w). The discrepancy observed is probably related to the higher amount of water added in the latter study, which presumably caused a major proportion of 3,4-DHPEA-EDA to dissolve in the water phase, which impoverished the oil. 3,4-DHPEA-EDA has antioxidant properties similar to oleuropein and stronger antioxidant properties than hydroxytyrosol (Paiva-Martins et al., 2009) due to its o-diphenolic structure and the lack of the –COOCH3 group which is not an electron donor group. Therefore, it was hypothesized that the observed increase in 3,4-DHPEA-EDA in 'Leccino' oil obtained with 5% water addition (Table 1) could improve its oxidation stability. 3,4-DHPEA-EDA has been considered as the intermediate compound in an alternative biosynthetic pathway leading to the formation of oleuropein (Alagna et al., 2012). Despite that, the concentration of oleuropein + ligstroside aglycones I & II and oleuropein aglycone (isomer II) decreased in the oils obtained with water addition (Table 1), which was in agreement with the results of (Kiritsakis et al., 2017) in the case of 'Koroneiki' cv. oil obtained by the addition of 25% w/w water. On the other hand, the water addition did not affect the concentration of ligstroside aglycon (isomer III). Considering higher antioxidant properties of secoiridoides compared to other classes of phenolic compounds (Carrasco-Pancorbo et al., 2005; Tripoli et al., 2005; Servili et al., 2015; Bouaziz et al., 2005), it can be assumed that a higher amount of secoiridoides in L-W samples could contribute to higher antioxidant activity and better stability of these oils.

Total flavonoids increased after the addition of water during olive paste malaxation by 22% regarding control oil due to the increase in the concentration of luteolin (Table 1). Luteolin has been reported as a phenolic compound with good antioxidant properties but with lover antioxidant activity than hydroxytyrosol and oleuropein (Bouaziz et al., 2005). On the other hand, phenolic acids (vanilic and *p*-cumaric acids) were not affected by the addition of water to olive paste during malaxation phase (Table 1), which is in agreement with the results of Kiritsakis et al. (2017) in the case of vanillic acid despite higher water volume that was added. Considering lignans, 5% (w/w) of water addition also had no influence on acetoxypinoresinol and pinoresinol concentration in 'Leccino' oil (Table 1), probably due to lignans' lipophilic character and low antioxidant activity, which makes them less dependent on partitioning and oxidation (Parenti et al., 2008).

Conclusions

Obtained results pointed out that the addition of a small amount of water (5% w/w) during malaxation had no significant influence on oil yield and extractability index of 'Leccino' cultivar nor on the total phenolic compounds in the obtained oil, while the phenolic profile was significantly affected. Observed changes in the phenolic profile included an increase in total secoiridoids, 3,4-DHPEA-EDA and flavonoids, which indicates the possibility of positive influence on oil oxidative stability, and consequently extension of the shelf life of oil. Moreover, further research, including olive fruits of different cultivars with different fruit characteristics regarding initial moisture content in the fruit and different ranges of water added during the extraction process as well as shelf life studies of obtained oils, are desired to increase the knowledge about the influence of water addition to the oxidative stability and quality of the final product.

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