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PHENOLIC, TOCOPHEROLS AND SQUALENE PROFILES (HPLC-UV) OF *CHEMLALI*-SFAX OLIVE OIL ACCORDING TO EXTRACTION PROCEDURE

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The aim of this work was to evaluate the impact of the microwave treatment as well as the effect of sodium citrate as co-adjuvant on legal parameters. The addition of microwaves (72 seconds, followed by 20 minutes of malaxation) results a low oxidation of olive oil and, consequently, a reduction in the peroxide value compared with the conventional method. Microwave processing was therefore confirmed as an attractive alternative to the conventional malaxation, with the main advantages being the rapid processing time and the high olive oil quality. On the other hand, the combination of microwaves and sodium citrate as co-adjuvant tested in two concentrations followed by a malaxation step for 20 minutes gave good results in term of polyphenols content compared with the conventional treatment (PT1, which correspond to traditional condition, *i.e*, 40 min of malaxation). The results clearly showed the efficiency of both treatments on olive paste to obtain oil having the characteristic of extra virgin olive oil.
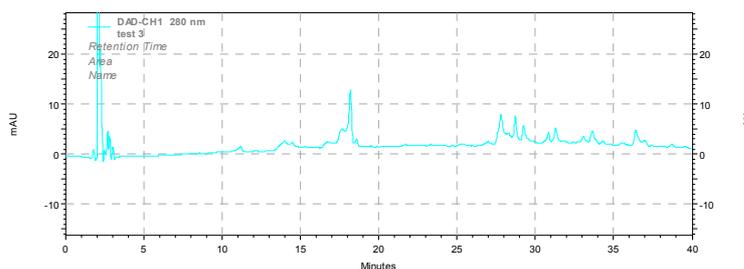
INTRODUCTION

Olive oil is part and parcel of the cultural and historical humanity heritage. This organoleptic and nutritional product is served in the great occasions, recommended by great chefs and studied in the scientific field. Tunisia occupies the fourth rank as producer and the second as exporter of olive oil in the world. Sixty percent (60%) of the Tunisian production are obtained from the *Chemlali* cultivar and the rest from other varieties.[1] In general, olive oil is composed of 99% of triglycerides. Its composition of fatty acids is mainly characterized by mono-unsaturated fatty acids, particularly oleic acid, and poly-unsaturated fatty acids (linoleic and linolenic acids). Other interesting features of olive oil definitely reside in its richness with minor compounds, such as phenolic compounds that are the major components responsible for the nutritional benefits of olive products.[2] In fact, phenolic compounds represent approximately 2% of fresh fruit.[1] The most important classes of these compounds are phenolic acids such as ferulic and vanillic acids, phenolic alcohols like hydroxytyrosol and tyrosol, flavonoids and secoiridoids such as oleuropein.[3] The most important compounds regarding the sensory quality of virgin olive oil are polyphenols and volatile compounds.[2] The quality of

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olive oil is dependent on the extraction method. The malaxation is one of the parameters that can reduce the viscosity of oil and subsequently facilitate the extraction phenomenon. The traditional malaxation time varied between 30 and 40 minutes.[4] This long period of time contributes to the oil oxidation and subsequently to its lower quality. To improve quantitatively and qualitatively the extraction of olive oil, addition of innovative techniques can be used before or during the process of malaxation. Therefore, microwave is a new technology that is used in many food processing applications, which has led to significant improvements in these processes. This new technology may increase production efficiency of oil, quality indexes and contribute to environmental preservation by reducing the use of water and solvents, elimination of wastewater.[5] The inherent principle of microwave heating is based on the transformation of electromagnetic energy into thermal energy through the immediate interaction of the former with polar molecules in a reaction mixture. The use of microwaves allows reducing the malaxation time while improving the quality of the olive oil.[6] Fiori *et al.*[7] showed that 72 seconds of microwaves corresponded to the optimum time to improve the quality of olive oil. Several other studies have shown that the addition of a co-adjuvant during the malaxation step can increase the concentration of phenolic compounds.[4-8] Various co-adjuvants such as micronized talc,[2] citric acid,[8,9] sodium carbonate [4] and sodium hydroxide (NaOH)[10] have already been used. In this work, we used, for the first time, the sodium citrate as co-adjuvant because it is the conjugate base of citric acid and also can help to improve the quality of oil. It is prepared by neutralizing citric acid with sodium hydroxide or sodium carbonate. Sodium citrate is an additive that has an antioxidant effect (E331). The combined effects of three malaxation parameters (time, microwaves and sodium citrate concentration as co-adjuvant) on *Chemlali* olive oil yield and quality were studied. Several analytical methods, legally established, were selected to evaluate the quality of the extra virgin olive oil (EVOO): Extraction yields, quality indexes, total phenols, chlorophylls and carotenoids pigments, squalene and α-tocopherols contents were determined.

MATERIALS AND METHODS

Olive fruit variety

In this work, olives from the variety *Chemlali* (Sfax, south Tunisia) were studied. The olives were harvested during the crop season 2014-2015. The maturity index was determined according to the method developed by the Agronomic Station of Jaén[1] as a function of fruit color (epicarp and mesocarp). For this study, a maturity index, equal to 5, was determined on 100 randomly selected olives trees. After harvesting, the olives were transported on the same day to the laboratory.

Preparation of olive pastes

After leaf-removal and olive washing, olives of *Chemlali* variety were divided in six homogeneous batches of 1 kg each. For laboratory scale simulation of virgin olive oil industrial process, the paste was mixed with water (0.5 L/kg of fresh fruits) [11] and the oil was extracted using an Abencor system (Comercial Abengoa, S.A., Seville, Spain). In the Table 2, the temperature of the olive paste was measured immediately after microwave exposure, by inserting a thermocouple (K-type; Ni/Al-Ni/Cr) connected to an acquisition system (HI 98804, Hanna Instrument, Villafranca Padovana-PD, Italy) at approximately the geometrical centre of the sample. Olive oil was filled in dark coloured glass bottles without headspace filled under nitrogen flux. The samples were kept at +4 °C in refrigerator. Experiments were performed in triplicate (Table 1). No control was carried out using a short malaxation time (20 min), as it is widely known that malaxation times lower than 30 min, without additional strategies such as the use of co-adjuvants or the increase of temperature, give losses in the oil yield.[12] Microwave treatment was carried out using a microwave oven (DELONGHI, Reference: 533 MW, processing time: 72 seconds, power: 900 watt). Microwave energy (E) was calculated according to the Buffler method[13] using the equation E= Wt/m, where, W is microwave oven power, t is the time of microwave exposure, and m = quantity of sample. Furthermore, the energy given to olive paste for all studied sample were equal to 64.8 kJ/kg.

Extraction of olive oil

The solid phase (olive pomace) of the paste was eliminated. The liquid phase (wastewater and oil) was then decanted for one hour. Finally, the supernatant (oil) was then removed using a syringe. The oil samples were stored at 4 °C in the dark before analysis. [12]

Table 1

Different treatments realized on olive pastes

	Without treatment	Treatments			
	Controls	Microwaves (s)	Sodium citrate (g/L)		Microwaves 72s + Sodium citrate (g/L)	
Treatment conditions	-	72	150	300	150	300
Malaxation time (min)	40	20	40	40	20	20
Sample abbreviation	PT1	PT2	PT3	PT4	PT5	PT6

Quality indices

Free acidity (% oleic acid), peroxide values (meq O_2/kg VOO), and ultra-violet absorption at 232 and 270 nm (K_{232} and K_{270}) were measured following the analytical methods described previously.[13-15]

Chlorophylls and Carotenoids contents

The chlorophyll and carotenoid contents (mg/kg of oil) were determined in cyclohexane. These parameters were then analyzed using a UV-Vis spectrophotometer (Lasany Model I-290) at 670 nm and 470 nm for chlorophyll and carotenoid, respectively.[16]

Analysis of α-tocopherol and squalene

Chromatographic purification of α-tocopherol and squalene

The procedure of squalene and α-tocopherol purification from olive oil was optimized as follows: Olive oil (1 g) was weighted and dissolved in 0.5 mL of *n*-hexane. The column on silica gel (1.5 x 30 cm, 0.2 mm, 70-230 mesh ASTM, Scharlau Chemie SA (Spain)) was conditioned with 60 mL of *n*-hexane, before adding the solution oil. Squalene (Fraction A) was eluted with 150 mL of *n*-hexane while α-tocopherol was eluted with 2 x150 mL of *n*-hexane-diethylether (99:1; v/v) (Fraction B). The collected fractions were evaporated under reduced pressure at room temperature. The purity and the concentration of the fractions of squalene and α-tocopherol were controlled by HPLC analysis.[1]

Determination of α-tocopherol and squalene contents

α-tocopherol concentration was determined by high-performance liquid chromatography (HPLC) according to the EEC.[14] An accurate amount of olive oil (2 g) was mixed in 25 mL of cyclohexane and homogenized. Sample preparation was conducted in dark until use. Amounts of α-tocopherol were measured by Perkin Elmer apparatus, using a Series 200 pump (USA) equipped with a Series 200 UV/Vis detector (USA). The chromatographic separation was achieved by a C-18 Hewlett-Packard ODS Hypersil column (5 μm particle size, 100 × 2.1 mm) operating at 32 °C. The mobile phase used was a mixture of methanol-CH_3COONa 0.05 M, pH 5.5 (95:5, v/v) at a flow rate of 300 μL/min, and the injection volume was 20 μL. The detection was carried out to 290 nm. The concentration of the solution (in mg of α-tocopherol per kg of olive oil) is calculated thanks to the calibration curve (Peak area = 9835 [C], $r^2 = 0.862$).

The squalene content was determined by diluting 0.5 mL of olive oil in 1 mL *n*-hexane and by analyzing the solution by the same HPLC apparatus.[1] The mobile phase was changed to be an isocratic phase (100% acetonitrile) while the flow rate was 1.2 mL/min. The detection was carried out at 208 nm. The compounds were identified by chromatographic comparisons as standards. Squalene concentrations were then calculated from integrated peak areas of the samples and the calibration curve of squalene. Good linearity was achieved in the range 187-600 mg/kg (Peak Area = 18490 [C], $r^2 = 1$).

Table 2

Quality indices of oils extracted from different paste treatments

Quality indices / Paste treatments	Free acidity (%)	Peroxide value (meq.O_2/kg)	K_{270}	K_{232}	α-tocopherol (mg/kg)	Squalene (mg/kg)	T° (C) after treatment
PT1	0.22 ± 0.01^a	6.52 ± 0.32^a	0.04 ± 0.00^a	2.00 ± 0.10^a	576 ± 28.80^a	1217 ± 61.85^a	17.9 ± 0.4^a
PT2	0.11 ± 0.00^b	3.26 ± 0.16^b	0.003 ± 0.00^b	0.95 ± 0.04^b	374 ± 18.70^b	2174 ± 108.70^b	20.8 ± 0.3^b
PT3	0.33 ± 0.01^c	3.26 ± 0.16^b	0.07 ± 0.00^c	0.68 ± 0.03^c	211 ± 10.50^c	2152 ± 107.60^b	15.3 ± 0.6^c
PT4	0.28 ± 0.01^d	5.43 ± 0.27^c	0.13 ± 0.00^d	0.81 ± 0.04^d	490 ± 24.50^d	2239 ± 111.95^b	15.9 ± 0.1^c
PT5	0.33 ± 0.01^c	3.26 ± 0.16^b	0.04 ± 0.00^e	0.37 ± 0.05^e	45.6 ± 2.28^e	1554 ± 77.70^c	16.1 ± 0.2^c
PT6	0.28 ± 0.01^d	4.24 ± 0.21^d	0.08 ± 0.03^f	0.70 ± 0.04^c	156 ± 7.80^f	2315 ± 115.75^b	16.2 ± 0.2^c

Means with different letters in the same column were significantly different at $p<0.05$. Results are expressed as mean± standard deviation of 3 determinations.

Analysis of phenolic compounds

Extraction of the phenolic fraction from olive oil

The phenol extracts were obtained as described before by Rigane *et al*.[1] The hydroalcoholic fractions were gathered together, washed with 4 mL of n-hexane to eliminate the residual oil, and then dried by evaporative centrifugation under vacuum at 35 °C.

Total phenols content

The amount of total polyphenols was determined using the Folin–Ciocalteu's method according to the methods described previously by Yahyaoui *et al*. [17] Total phenol content was expressed as mg gallic acid per gram of ethanolic extract (mg GAE/g extract). All samples were analyzed in triplicate.

Reverse-phase HPLC conditions for phenolic compounds

The HPLC analysis were performed with a Schimadzu apparatus composed of a LC-10AT pump, a SPD-10A UV–vis detector using a Shimpack C-18 column (250 × 4.6 mm, particle size 5 μm) from Shimadzu company (Kyoto, Japan). The flow rate was 0.5 mL/min. The mobile phase was 0.1% phosphoric acid in water (A) and 70 % acetonitrile in water (B). The solvent gradient started with 20% B and increased to 50% (B) in 30 minutes, followed by an isocratic plateau for 5 minutes. Finally, solvent B decreased to 20% until the end of running time.[1] The injection volume was 20 μL. The temperature was maintained at 40 °C. All samples were filtered through a 0.45 μm Minisart filter before injection.

Detection and quantification were performed at 280 and 320 nm. Each phenolic compound was expressed with its standard when it was available. Eight relevant standards were selected and tested to be quantified in our samples: tyrosol (one of the main antioxidants in olive oil), catechol, gallic acid, caffeic acid, vanillic acid, ferulic acid, *p*-coumaric acid and quercetin. The concentration of the solution (in μg of standards per g of olive oil) is calculated thanks to the calibration curves.

Statistical analysis

Results of the analytical determinations were expressed as mean ± standard deviation (SD) of three measurements. Statistical differences were calculated using a one-way analysis of variance (ANOVA), employing the Student's t-test. Differences were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Extraction yields

The oil extraction yield is one of the most important economical parameters for the grower. In order to improve this parameter, many malaxation conditions can be varied (malaxation time, paste treatment with a co-adjuvant and use of microwaves). The extraction yields obtained for PT1 and PT2 were both equal to 66 % (Figure 1). This result indicates that the use of microwaves for 72 seconds followed by a 20 minutes malaxation is equivalent to a single malaxation of 40 minutes. Conditioning olive paste by using an industrial-sized microwave assisted apparatus instead of the conventional malaxation process has recently been reported.[6,18,19] In these studies, no significant differences were found regarding the extraction yields. Microwave processing was therefore confirmed as an attractive alternative to the conventional malaxation; the rapidity of the process being the main advantage. On the other hand, sodium citrate was used for the first time as co-adjuvant in the extraction of olive oil. This co-adjuvant is the conjugated base of citric acid. The addition of sodium citrate at 150 g/L or 300 g/L, combined or not with microwave treatment (PT3 to PT6), slightly decreases the obtained extraction yields compared with the corresponding controls (PT1 and PT2). Chih *et al*.[9] who used another co-adjuvant, mentioned that the citric acid particles could effectively rupture the cell at microscopic level, but the different concentrations of co-adjuvant did not modify the extraction yields of olive oil. Figure 1 also shows no interaction effect between malaxation time and addition of co-adjuvant (PT3, PT4, PT5 and PT6) as mentioned by Espinola *et al*.[2] It is the first time that microwave treatment and co-adjuvant as sodium citrate was combined. There are no significant differences on the extraction yields of olive oil (PT3 and PT4 compared with PT5 and PT6).
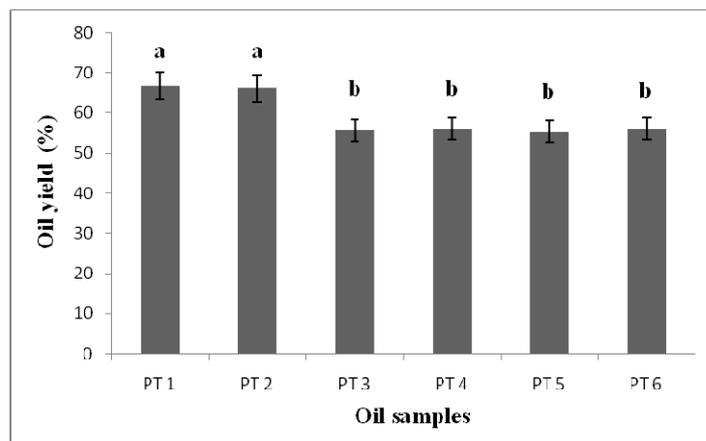
Fig. 1 – Oil extraction yields (%) obtained after different paste treatments (PT). Means with different letters were significantly different at $p<0.05$. Results are expressed as mean ± standard deviation of 3 determinations.

Quality parameters

Free acidity is an index that determines quality parameters of olive oil from an analytical point of view. Acidity measures free fatty acid quantity present in oil.[20] It should be noted that extra virgin olive oil should have a maximum free acidity, expressed in oleic acid, of 0.8%. From Table 2, we can conclude that the treatment of olive paste by microwave decreases the free acidity of oil (PT1 and PT2: 0.22 % and 0.11 %, respectively). This observation resulting from laboratory experiments is not in accordance with Tamborrino *et al.*[18] who mentioned that there is no significant difference observed for free acidity between olive pastes conditioned using the microwave-assisted treatment on large-scale production, and olive paste traditionally treated. Compared to the traditional method of malaxation (PT1) and to the microwave treatment (PT2), the addition of sodium citrate associated or not with microwaves increases the acidity index. This increase of acidity could be explained by triglycerides hydrolyses reactions.[21] Hence, the combination of microwaves and sodium citrate at different concentrations (150 g/L or 300 g/L) did not modify the oil concentration in free acidity (PT5 and PT6: 0.33% and 0.28%, respectively). Finally, we can conclude that in all cases, all oils have the characteristics of extra virgin oil. In addition, peroxide value allows us to make an estimation of oxygen quantity necessary for the degradation of olive oils. Olive oil peroxide index obtained from PT1 corresponds to 6.52 meq.O_2/kg (Table 2). In all other experiments, olive oils displayed lower peroxide indexes than those obtained with the traditional treatment of malaxation and all oils produced there have the

same characteristics of an extra virgin oil according to EEC.[14] The effect of microwaves could certainly be ascribed to weak contact with atmospheric oxygen during malaxation, which prevents lipid auto-oxidation reactions resulting from peroxide formation decrease.[18] The results obtained in this study are similar to those obtained by Malheiro *et al.*[15] who studied the effects of microwaves on the oil with different durations of malaxation on the peroxide index. They mentioned that the peroxide values behaviour of extra virgin olive oils could be explained by changes during oxidation process, with this value reaching a maximum due to hydroperoxides formation, and then decreasing due to the appearance of secondary products. In addition, PT2 and PT3 have similar indexes (3.26 meq.O_2/kg), showing that a treatment of the paste, during 72 seconds of microwaves followed by 20 minutes of mixing is comparable to 40 minutes of malaxation in the presence of a solution containing 150 g/L of sodium citrate. The additions of the two new factors (microwave and co-adjuvant) do not improve the peroxide index which remains identical to the previous values (PT5 compared to PT2 and PT3). The comparisons between experiments PT3, PT4 and PT5, PT6 indicate that the peroxide index with the highest concentration of sodium citrate (300 g/L) was higher than that obtained with the lowest concentration (150 g/L). The results of the present study are in contradiction with Chih *et al.*[9]. These latter, found a maximum of peroxide index with 150 g/L of citric acid as co-adjuvant. On the other hand, our research team studied the K_{232} and K_{270} which are measured from absorptions at 232 and 270 nm respectively, with a UV spectrophotometer by dissolving the sample in

cyclohexane. These coefficients are quantified to determine the presence of conjugated diene and triene systems resulting from oxidation processes.[15] The European Union commission Regulation[14] considers that "extra virgin olive oil" should not exceed: 2.5 for K_{232} and 0.2 for K_{270} to be branded. The treatment of the olive paste, either by microwaves, by addition of sodium citrate or by the combination of both (microwave and co-adjuvant addition), causes in almost all cases a decrease in the coefficients K_{270} and K_{232} compared to those obtained by the traditional method of malaxation (PT1) as shown in Table 2 except certain treatment for example (PT4) increase in the coefficient K_{270} in a remarkable way. Similar results were obtained by Malheiro *et al.*[15] with microwaves (PT2). Reboredo-Rodriguez *et al.*[22] revealed that the more the malaxation times were important the more the K_{270} and K_{232} coefficients were high. These decreases could be attributed to the intensification of the primary and secondary oxidation process (Table 2). Therefore, from these data, we can conclude that all oils have the characteristics of an extra virgin oil, according to EEC.[14]

Chlorophylls and carotenoids content

Olive oil contains minor compounds that give it its organoleptic and nutritional qualities. Among these minor compounds, pigments, due to their antioxidant characteristics in obscurity and pro-oxidant ones in light, seem to play an important role in the stability of oxidation in the olive oil during storage and in the preservation of its quality.[23] Chlorophyll pigments are present in olive oil and are responsible for the greenish coloration of certain oils.[24] In Figure 2a, we can note that microwave treatment as well as the addition of sodium citrate at 300 g/L increased chlorophylls levels of PT2 and PT4 (1.72 and 1.57 mg/kg, respectively). These results are in accordance with Malheiro *et al.*[15]. Cruz *et al.*[25] who reported that oils obtained from Manzanilla variety olives using common salt as co-adjuvant show significant increase on the chlorophyll content. In contrast, a significant decrease was observed in chlorophylls levels when the two treatments were combined PT5 and PT6 (0.45 and 0.38 mg/kg, respectively), or when using sodium citrate at 150 g/L was used PT3 (0.64 mg/kg). In these cases, color loss olive oils were remarkably noted, seemingly, due to chlorophylls loss. Consequently, this quickens degradation speed of olive oil and enables radical

peroxids formation. The carotenoids behavior was almost similar to chlorophyll (Figure 2a).

α-tocopherols levels

Tocopherols are important compounds of olive oil because of their contribution on the high quality of the product. In virgin olive oil, α-tocopherol is considered as the major fraction in tocopherols which may reach 95%. In addition, it was considered as an important antioxidant.[15] Tocopherols constitute the lipophilic antioxidant group and are well known for their effective inhibition of lipid oxidation in all vegetable oils. The α-tocopherol contents for different olive oils obtained after microwaves treatment and/or citrate sodium solutions are observed in Table 2. Compared with the α-tocopherol content obtained by the traditional method PT1 (576 mg/kg), all treatments decrease the concentration of this compound (PT2 to PT6). The decrease of their content in the olive oils obtained by the insertion of new technologies could be explained by the fact that a quantity of α-tocopherol has already been consumed to protect olive oil from oxidation. A weak concentration of α-tocopherol may equally reduce conservation time of olive oils. In addition, as the variety of olive *Chemlali* Sfax has the higher content of α-tocopherol in Tunisia,[1] the decrease of the α-tocopherol concentration is not a major problem for oil conservation.

Squalene levels

Squalene acts as a weak antioxidant in olive oil. The presence of squalene in virgin olive oil has a protective effect on the oxidative stability under heating. Besides, the squalene concentration varies widely depending on the extraction procedure of olive oil, with a range of 2 to 7 g/kg of oil.[26] Contrary to the results obtained with α-tocopherol and in comparison, with the traditional method PT1 (1217 mg/kg), all the treatments increase the concentration of the squalene antioxidant (Table 2). The results indicate that a very weak difference of squalene content is present in olive oil supplied by olive pastes which have been treated by microwave and/or the addition of an aqueous citrate sodium solution excepted paste treatment PT5 (1554 mg/kg). All the antioxidants quantity is related to various endogenous enzymes activities such as pectinases, hemi-cellulases and cellulases present in olive oil that are probably activated during extraction process and storage.[27]
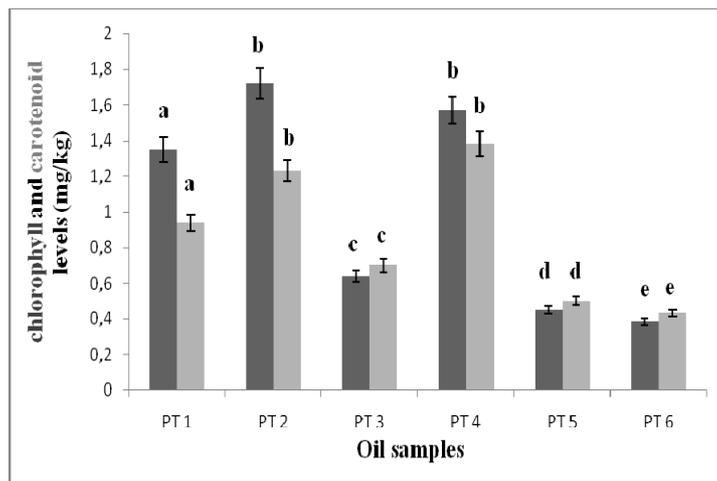
Fig. 2a – Chlorophyll and carotenoid contents in olive oil extracted from olive pastes with different treatments. Means with different letters with the same color were significantly different at $p<0.05$. Results are expressed as mean ± standard deviation of 3 determinations.
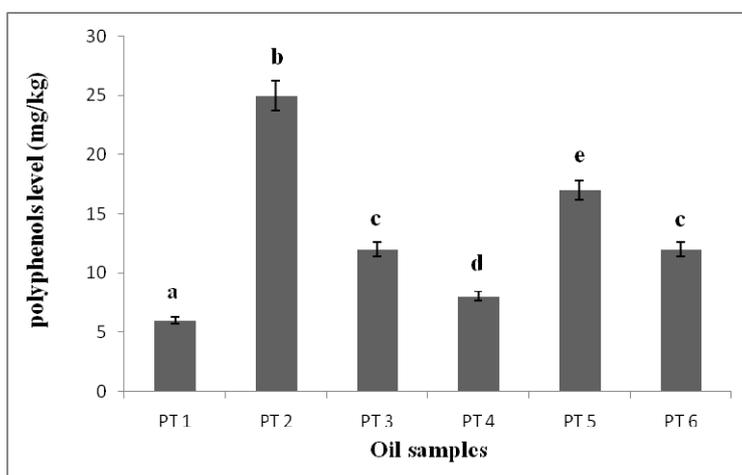

Fig. 2b – Polyphenols content of oils resulting from different treatments. Means with different letters were significantly different at $p<0.05$. Results are expressed as mean ± standard deviation of 3 determinations.

Concentration of total phenolic compounds

The importance of phenolic compounds in virgin olive oil is mainly attributed to their action as natural antioxidants, which may contribute to the prevention of several human diseases. Besides, phenolic compounds are responsible for the shelf-life of the oils and for their typical bitter taste.[28]

As shown in Figure 2b, microwave treatment and/or the addition of sodium citrate associated with a decrease in malaxation time increase the content of total phenol in olive oil. The highest concentration of phenolic compounds is obtained after treatment with microwaves only PT2 (25 mg/kg). After the addition of sodium citrate, the phenolic compounds content is also increased compared with the traditional method PT1 (6 mg/kg). A higher concentration of phenolic compounds is obtained with the lowest concentration of co-adjuvant PT3 and PT4 (12 and 8 mg/kg, respectively). The combination of microwaves and

co-adjuvant PT5 and PT6 (17 and 12 mg/kg, respectively) gives concentrations of phenolic compounds intermediate between the levels obtained with microwaves alone (PT2) and the addition of sodium citrate only (PT3 and PT4). Malaxing time affects significantly phenolic content.[29] Aliakbarian *et al.*[8] used citric acid as co-adjuvant and they showed that the enhancement in the total content of polyphenols was attributed to both rissing citric acid and malaxation time. In addition, Vierhuis *et al.*[30] mentioned that the main enzymes, involved in the liberation of phenol in olive oil, are pectinases endogenes, hemi-cellulases and cellulases that hydrolyze cellular wall. Therefore, Di Giovacchino *et al.*[31] have demonstrated that the extraction technique might affect total phenolic content concentration in olive oil and, subsequently, its bioactivity. The rise on phenolic content level is probably assigned to different mechanisms exerted by microwave treatment. One of which consists of the inhibition of

polyphenol oxidase, an enzyme which decomposes phenolic compounds. This requires a high level on microwave in order to break the cellular structure, inhibit polyphenol oxidase activity and enable phenolic compounds migration to the oily phase. Finally, these technologies may disrupt biological cell walls, facilitating the release of minor compounds.[32]

Qualitative results of the olive oil extraction

The quantification of phenolic compounds of olive oil samples was performed by RP-HPLC: the averaged concentrations, expressed as μg/kg of olive oil (Fig 3), with the prevalent presence of phenolic alcohols, phenolic acids and flavonoids, are reported in Table 3. Tyrosol, an important phenolic alcohol in the olive oil, is present in high levels in PT3 to PT6 (365 to 793 μg/kg, respectively) in which we used citrate sodium as co-adjuvant. These results could be explained by the rupture of some complex molecules containing tyrosol such as nuzhenide and ligstroside.[33] Based on the results in Table 3, we can conclude that the use of microwaves and/or sodium citrate treatments (PT2 to PT6) increase the total concentration of the phenolic acids. Gallic acid is present in very high concentrations but the other compounds, as vanillic, caffeic, *p*-coumaric and ferulic acids, are present in very low concentrations. These results were confirmed by Tsimidou,[34] who reported the occurrence of phenolic acids as minor components of virgin olive oil. The prevalent flavonoid is quercetin. Its content in traditional treatment (PT1) is equal to 525 μg/kg of oil. The use of microwave treatment of olive paste (PT2) causes a decrease on the amount of this flavonoid compound (228 μg/kg of oil) while the addition of citrate sodium (150 and 300 g/L) on the olive paste (PT3 and PT4) during olive oil extraction without microwave treatment allows to increase the quercetin level (776 and 1743 μg/kg of oil, respectively). The microwaves treatment with 300 g/L or 150 g/L of sodium citrate aqueous solution, on olive paste and a malaxation for 20 minutes (PT5 and PT6) allows to increase the quercetin content to be 750 and 948 μg/kg of oil, respectively. In fact, the addition of sodium citrate to the olive paste increases the phenolic content of the studied samples. The presence of co-adjuvant could facilitate the dissolution of phenols in the oil, inducing an increase of both stability against oxidation and the bitter taste.[35] These results are in agreement with those reported by Fiori *et al.*[7] who found that

optimal operative conditions applied during malaxation after 72 seconds followed by microwaves treatment at 20 minutes malaxation of the olive paste can be opportunely chosen for improving the relative virgin olive oil quality.

CONCLUSION

The results of the present work degree focus on the implementation of olive oil nutritional quality amelioration and in the second time, thanks to innovative technologies introduced on the olive paste able to reduce the wastewater rejected in the wild. They brought about advances on several levels. To start with the introduction of microwave treatment of olive paste, during olive oil process extraction allowed a quick heating of olive paste and a considerable reduction in treatment time which caused a decrease in olive oil oxidation, when compared to traditional procedure. The phenolic content increases, reaching 25 mg/g of olive oil by adding microwave in the 20 minutes of malaxing compared to other paste treatments. This was found by quality parameters results, which, accordingly, would ameliorate olive oil quality. The use of these data, in these definite conditions, will allow an extraction of extra virgin olive oil. The second important advance is concerned with the addition of co-adjuvant as sodium citrate aqueous solution in two concentrations (150 and 300 g/L) on olive paste followed by microwave radiations treatment, yielding oils of extra virgin olive (EVOO). The addition of citrate sodium may eventually increase the quality of olive compared to conventional extraction (PT1). With the exception of peroxide values and α-tocopherol, all parameters are significantly affected by the addition of microwaves and sodium citrate. These two impacts (microwaves and sodium citrate) are beneficial because they enrich the quality of olive oil in terms of total polyphenols, squalene, and allow amelioration in the efficiency of olive oil extraction. The results of this study will be useful in modification of present processes or development of new processes with the aim of protecting and improving the quality parameters as well as the phenolic composition of olive oil. From these data, we believe that describing the effects of microwaves as well as the addition of sodium citrate as a co-adjuvant on olive paste before malaxation will exhibit possible benefits for the future of olive processing technology. The optimization of olive oil extraction parameters with proper adjustments in such a way that the quality of the product is highly increased is an important issue.
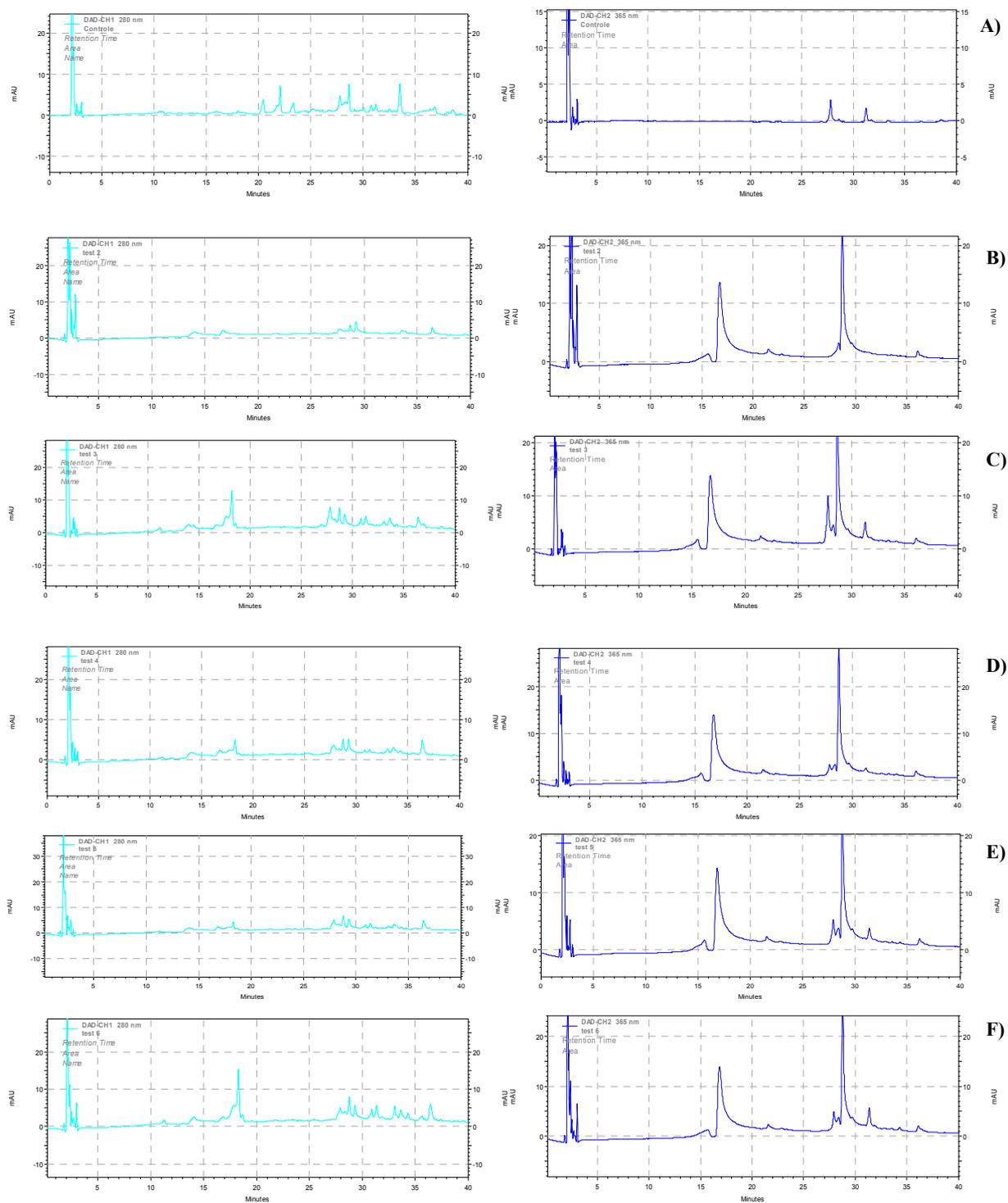
Fig. 3 – HPLC Chromatogramms of the phenolic compounds of each olive oil extracted using different treatments (λ= 280 nm and λ= 365 nm).

A) PT1 -> control; B) PT2 -> Test 2; C) PT3 -> Test 3; D) PT4 -> Test 4; E) PT5 -> Test 5;
F) PT6 -> Test 6

Phenolic compounds composition of the studied olive oils measured by HPLC.

Class of phenols	Compounds	T_R (min)[*]	Concentration of AOX (µg/kg of oil) of different treatments					
			PT1	PT2	PT3	PT4	PT5	PT6
phenolic alcohols	Gallic acid	2.7	926 ± 46.30^a	6403 ± 320.15^b	370 ± 18.50^c	3312 ± 165.60^d	1396 ± 69.80^e	937 ± 46.85^a
	Tyrosol	11.2	54 ± 2.70^a	ND	481 ± 24.05^b	774 ± 38.70^c	793 ± 39.65^c	365 ± 18.25^d
	Catechol	10.1	38 ± 1.90^a	ND	ND	102 ± 5.10^b	ND	ND
	Vanillic acid	12.7	68 ± 3.40^a	24 ± 1.20^b	5 ± 0.25^c	31 ± 1.55^d	47 ± 2.35^e	19 ± 0.95^f
Phenolic acids	Caffeic acid	13.7	8 ± 0.40^a	92 ± 4.60^b	219 ± 10.95^c	270 ± 13.50^d	349 ± 17.45^e	442 ± 22.10^f
	p-coumaric acid	16.9	ND	128 ± 6.40^a	156 ± 7.80^b	122 ± 6.10^a	20 ± 1.00^c	132 ± 6.60^a
	Ferulic acid	18.6	49 ± 2.45^a	ND	30 ± 1.50^b	88 ± 4.40^c	113 ± 5.65^d	43 ± 2.14^e
Flavonoids	Quercetin	27.2	526 ± 26.30^a	228 ± 11.40^b	777 ± 38.85^c	1743 ± 87.15^d	751 ± 37.55^c	948 ± 47.40^e

Means with different letters in the same line were significantly different at $p<0.05$. Results are expressed as mean± standard deviation of 3 determinations. *****: retention time in minutes; ND: not detected; AOX: antioxidant

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