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Microwave Heating of Different Commercial Tunisian Olive Oil: Regarding to Exposure Times on Physical and Chemical Parameters Properties

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Abstract

In this work the effect of different microwave heating times, that simulate the usual times used to cooking, on Tunisian olive oils was investigated. Traditional parameters, including free acidity, peroxide value, ultraviolet absorbance values at 232 and 270 nm, phenolic, chlorophyll and carotenoid compositions, were determined in four extra-virgin olive oil samples before and after microwave treatment. The results showed that heating by microwave apparatus produce losses in the quality of the different analyzed olive oils. The heating time did not promote the occurrence of hydrolysis in the samples since no changes in free acidity values were found. All other parameters were affected by exposure time in a similar way: in the first 3 min no marked changes were observed, after that the quality of the oil decrease significantly. Globally, the microwave heating time also affects the total chlorophylls, carotenoids and phenolic contents which clearly decreased as long as the exposure time increases. The use of extra-virgin olive oil may be encouraged especially at short microwave treatment times for both domestic and food catering applications.

Keywords: Olive oil; Microwave treatment; Phenolic compounds; Chlorophylls content

Introduction

Olive oil is conquering a key status in the worldwide cuisine, being one of the most important lipid sources in the Mediterranean diet. Besides its unique sensorial attributes, its beneficial healthy properties are increasingly documented: olive oil ingestion is associated with a lower incidence of some modern life-style diseases, including some kinds of cancer [1]. Beneficial health effects of olive oil are attributed to its high content of monounsaturated oleic acid as well as the presence of a myriad of biologically active minor components, which include a broad range of phenolic compounds, squalene, tocopherols, and sterols [2,3].

In the last decades, the modern life style brought procedures changes in the food and cooking processing technologies. Since its invention and development, the use of the microwave oven increased constantly, both at home and in the industry sector, due to its advantages, that includes capacity to rapidly transmit heat due to its high penetration power convenience, ease of use and time and energy savings oven is the tendency of the industry to produce pre-prepared food products especially to defrost, heat or cook using this kind of equipment [4-8].

The effect of olive time storage [9], olive fruit fly infestation [10], and olive ripening stage [11] on oil quality of Tunisian varietal olive oils was previously studied. Nevertheless, as far as we know, there is no investigation regarding the effect of microwave heating on Tunisian olive oils.

The objectives of this study were to verify the effect of different microwave heating times, 1,3,5,10 and 15 min, that simulate the usual times used to cooking, on four Tunisian olive oils one from the north "Chétoui", one from the centre "Oueslati", two from the south of Tunisia "Chemlali and Zalmati". The parameters used to evaluate this effect were free acidity, peroxide value (PV), specific extinction coefficients (K232 and K270), chlorophylls and carotenoids and phenols contents.

Practical Application

The application of the microwave oven heating to culinary techniques and food processing is a more recent development than other traditional cooking techniques. Advantages of microwave cooking include savings in time and energy and ease of use. These advantages make it one of the most attractive cooking methods.

Material and Methods

Chemical reagents

Methanol, n-hexane, cyclohexane and acetic acid HPLC-grade solvents were purchased from Riedel-deHaën (Buchs, Switzerland). Ethanol was obtained from Carlo Erba (Milan, Italy). The solvent were of appropriate purity. Folin–Ciocalteu (F-C) reagent was obtained from Fluka (Buchs, Switzerland).

Olive oil samples

Sufficient amounts of olives were hand-picked from all sides of different olive trees growing under rain-fed conditions during 2012/2013 crop season. The samples analyzed are Chemlali, Chétoui, Zalmati and Oueslati. The samples were immersed in liquid nitrogen, and stored at −80°C prior to analysis. The olive fruits (2 kg) were collected from olive tree plantations located in several localities in the

north, center and south of Tunisia. To obtain uniform amounts of fruits, collection was accomplished from different parts of each tree, so as to minimize the effect of watering and sun exposure. In order to eliminate the influence of maturation state on olive oil quality, the ripening degree was the same for all studied olive samples. The maturity index was determined according to the method developed by the Agronomic Station of Jaen as function of fruit color in both skin and pulp [12]. The maturity index was determined on 100 randomly selected olives from each sample.

Oil extraction

After harvesting, the olive samples were transported on the same day to the laboratory. Olive oil was obtained using an Abencor system (Comercial Abengoa, S.A., Seville, Spain). Olive fruits were washed, deleafed and milled into a paste in an electric mill, and the resulting paste was mixed for 30 min at 20°C and spun at 3500 rpm to obtain the oil. Samples were stored in amber glass bottles at a temperature of 4°C without headspace until analysis.

Heating procedure

To simulate conventional times used in home cooking, different times for microwave heating were selected: 1,3,5,10 and 15 min. For each olive oil and time, three sub-samples of 50 mL were individually placed in a Petri dish (20 mm high and 110 mm of diameter) and subjected to heating in a microwave oven (Kenwood) at maximum potency (1000 Watt). Unheated olive oil was used as control (corresponding to 0 min). Afterwards, the samples were kept in Falcon tubes and refrigerated until analysis.

Analytical methods

Quality parameters determination

Free acidity, peroxide value and coefficients of specific extinction at 232 and 270 nm (K232 and K270) were determined according to European Union standard methods [13].

Chlorophyll and carotenoid determination

Chlorophyll and carotenoid compounds ($mg.kg^{-1}$ of oil) were determined at 670 and 470 nm, respectively, in cyclohexane using the specific extinction values, by the method of Mínguez-Mosquera et al [14].

Chlorophyll ($mg.kg^{-1}$)=(A670 x 10^6)/(613 x 100 x d)

Carotenoid ($mg.kg^{-1}$)=(A470 x 10^6)/(2000 x 100 x d)

Where A is the absorbance and d is the path length of the cell (1 cm). The chlorophyll and carotenoid concentrations are expressed as mg of pheophytin α and lutein per kg of oil, respectively.

Analysis of phenolic compounds

Liquid–liquid extraction

The procedure was carried out using the analytical methodology described by Rigane et al. [3,15] with some modification. Briefly, the oil sample (4 g) was added to 2 mL of n-hexane and 4 mL of a methanol/water (60:40, v/v) solution in a 20 mL centrifuge tube. After vigorous mixing, they were centrifuged for 3 min. The hydroalcoholic phase was collected, and the hexanic phase was re-extracted twice with 4 mL of methanol/water (60:40, v/v) solution each time. Finally, the hydroalcoholic fractions were combined, washed with 4 mL of n-hexane to remove the residual oil, then concentrated and dried by evaporative centrifuge in vacuum at 35°C.

F-C Test for measurement of total phenol concentration

Briefly, 50 µL of phenolic extract was mixed with the Folin–Ciocalteau reagent (250 µL) and, with an aqueous solution of Na2CO3 (500 µL, 20%). The mixture was vortexed and diluted with water to final volume of 5 mL. The total phenol content was determined colorimetrically at 765 nm. The standard curve was prepared using diluted solutions of gallic acid in a methanol: water solution (70:30, v/v). The total phenolic content was expressed as milligrams of gallic acid (GA) equivalents per kilogram of oil. For gallic acid, the curve absorbance versus concentration was described by the equation y=0.095x (r^2=0.973) [3,15,16].

Statistical analysis

Results of the analytical determinations were expressed as mean ± standard deviation (SD) of 3 measurements. Statistical differences were calculated using a one-way analysis of variance (ANOVA), employing the Student's t-test. Differences were considered significant at $p < 0.05$.

Results and Discussion

Changes in free acidity

In the present work high quality olive oils were chosen from four commercial olive oils. Prior heating, all analyzed olive oils were classified as extra virgin olive oil, with free acidity value ranged from 0.52% to 0.75%. For the analyzed samples the free acidity values were similar at all the studied times (Figure 1) and no statistically changes were observed when its values were correlated with heating time. In certain way, the obtained results were expected and are in accordance with the results obtained by Albi et al [17] and Malheiro et al [5].

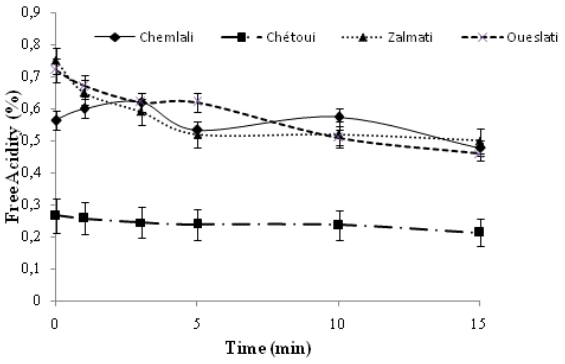

Figure 1: Changes occurred in free acidity values (% of free fatty acids) of Chemlali, Chétoui, Zalmati and Oueslati subjected to different microwave heating times.

Free acidity values results from the occurrence of hydrolysis in fatty acids. In those reactions hydrolytic enzymes are involved. Normally, these enzymes are presents in the olive fruit or surrounding microorganisms. In the present work we only use high quality olive

oils that are filtered and dehydrated, the probability of enzymes occurrence is low or inexistent [5,17].

Changes in peroxide value

Peroxide values (PV) were used for an estimation of oxidative degradation. Before microwave heating, the PV of the olive oils analyzed was between 2.64 and 8.08 med.kg^{-1} being lower than the maximum values indicated by the EEC Regulations [13]. However, when the Chemlali and Zalmati olive oils were heated until 15 min, a higher peroxides was observed, which overcoming the maximum permitted limit, and consequently losing the classification of virgin olive oil category (Figure 2).

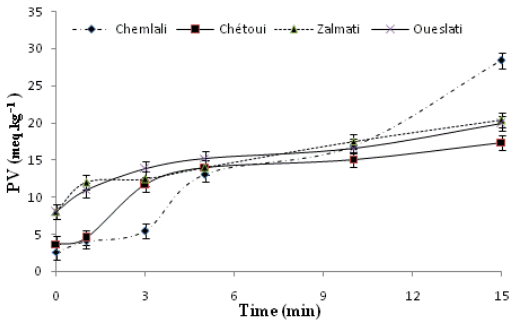

Figure 2: Changes occurred in Peroxide Value (PV) of Chemlali, Chétoui, Zalmati and Oueslati subjected to different microwave heating times.

Exposure to microwave energy was reported to favor the formation of free radicals [19]. However, peroxides usually undergo further degradation not only at high but also at low temperature [20]. The peroxide value behavior of extra olive oils could be explained by changes during oxidation process, reaching this value a maximum due to hydroperoxides formation, and remained approximately constant until the end of the heating treatment (Figure 2). This may be related to the fast heating due to microwave exposure, which has both decreased the oxygen availability and shifted the oxidation reaction balance towards a greater formation of secondary oxidation products (Figure 2).

Literature reports a small peroxide value increase in extra virgin olive oil after microwave heating at 170°C for 120 min [17], while, Cossignani et al [21] reported a peroxide value increase in extra virgin olive oil after microwave heating exposure during 8 min at medium-power. On the other hand, Malheiro et al [5] mentioned that the peroxide value of Azeite de Trás-os-Montes PDO and Azeite da Beira Interior PDO, two Portuguese olive oils, decreased when treatment by microwaves heating was up to 10 min.

Changes in specific extinction coefficient at 232 nm and 270 nm

With the evaluation of the specific extinction coefficients (K232 and K270) it is also possible to verify the degree of olive oil oxidation, complementing the observations for the PV. These specific extinction coefficients are indicative of the conjugation of trienes (K232) and the presence of carbonyl compounds (K270), respectively [3,5,15]. The maximum values permitted for K232 and K270 are respectively 2.50 and 0.20 for extra virgin olive oils.

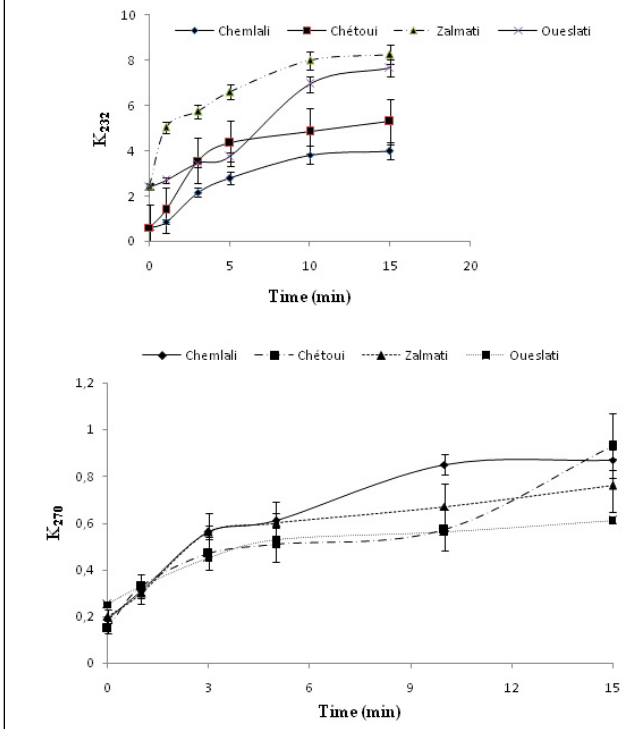

Figure 3: Changes occurred in K232 and K270 of Chemlali, Chétoui, Zalmati and Oueslati subjected to different microwave heating times.

Figure 3 shows changes in K232 and K270 specific coefficients under different exposure times at microwave heating. Before analysis the olive oils presented for all tested olive oil K232 values varied between 0.6 and 2.53, while K270 values ranged between 0.15 and 0.25. Before being heated all the parameters were within the legal limits [13]. Microwave heating brought significant changes in the K232 and K270 values, in all analyzed olive oils. All the UV parameters analyzed presented extremely positive correlation with exposition time. In the initial heating stages (approximately the first 3 min) the specific coefficient values showed little variations, after that a significant increase was observed for all the coefficients. At 15 min all oils presented a K232 higher than 4 that indicates an accelerated degradation process. The same situation was also observed for K270 values (more than 0.6). These results were in accordance with those found by Albi et al [17]; Caponio et al [4] and Malheiro et al [5]. All these reports prove the worsening effect of microwave heating in olive oil for a long time (more than 5 min).

Changes in chlorophylls and carotenoids content

Chlorophylls are responsible for the greenish coloration of certain olive oils. Those pigments are also important in olive oil stability due to their antioxidant nature in the dark and pro-oxidant activity in the light [3,13,22]. At the beginning of the assay, their amount varied between 0.91 mg. kg^{-1} in the Oueslati olive oil and 5.75 mg.kg^{-1} in the Chétoui samples. Data presented in this study showed that a marked

decrease was observed in its levels for all the analyzed olive oils (Figure 4). This observation was more noticeable in the Chétoui olive oil.

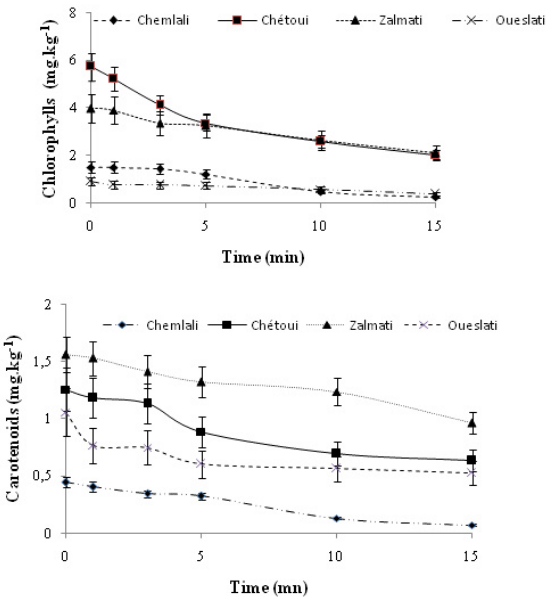

Figure 4: Changes occurred in Chlorophylls and Carotenoids content of Chemlali, Chétoui, Zalmati and Oueslati subjected to different microwave heating times.

Carotenes are present too in olive oil and are responsible of its yellow coloration [9]. Carotenoid content followed a similar trend to that of chlorophylls. The level of carotenoids in the unheated oils (time zero) ranged between 0.44 and 1.56 mg.kg^{-1} for Chemlali and Zalmati olive oils, respectively. Figure 4 shows the changes occurred in carotenoid levels along the exposure times to microwave heating. The values remain practically constants until 5 min of heating and decrease drastically after that until 15 min.

These results were in agreement with those founded by Malheiro et al [5] who reported that the microwave heating time decreased the total chlorophylls and carotenoids contents as long as the exposure time increases.

Changes in phenolic content

Phenolic compounds are secondary metabolites that can be commonly found in many plants [3]. Currently, these compounds are receiving considerable attention because its antioxidant activity, strongly related to cancer prevention, inflammatory disorders and cardiovascular diseases.

To the authors' best knowledge; only one study [22] has reported the effect of microwave heating on phenols. Total phenolic content in the extra virgin oil samples was different: a medium-high amount of phenols was found and ranged between 73.22 and 359.26 mg.kg-1 of oil, respectively, for Oueslati and Chétoui olive oil. Phenolic compounds decreased in all studied oils with increasing microwave heating time as shown in Figure 5.

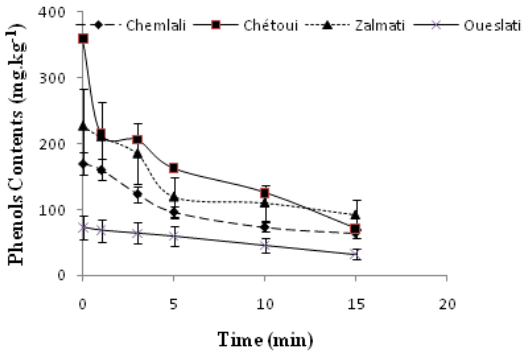

Figure 5: Changes occurred in phenolic content of Chemlali, Chétoui, Zalmati and Oueslati subjected to different microwave heating times

Total phenolic content of Chétoui olive oil showed a gradual reduction to reach ~162.47 mg.kg^{-1} of oil at 5 min of treatment (-55%), then a further decrease to 73.93 mg.kg-1 of oil (-80%) at the highest heating time. Phenols of all studied oil remained substantially unaltered at the lowest treatment times (1 and 3 min), but they showed a marked loss at 5 min of heating (more than 47%). A further decrease was observed thereafter, leading to low levels of phenols in well-known Tunisian olive oil after 15 min of microwave exposure.

Olive oil phenolic compounds were also evaluated by some authors in previous works. Generally, minor losses were reported after microwave heating for a short processing period. Brenes et al [23], using extra virgin olive oil and gentle processing conditions (10 min, 500 W), reported lower losses than those achieved during frying. In opposition, a huge decrease on total polyphenols in virgin olive oil and olive oil (>85%) was reported by Albi et al [24], for prolonged heating (120 min, 500 W). Cerretani et al [25], using high temperatures, reported similar decreases for extra virgin olive oil and virgin olive oil, particularly for more than 6 min heating (>255°C). These observations suggest that, for prolonged/intense microwave cooking, lower olive oil grades could present economic advantages, since most phenolic compounds are degraded. On the other hand, it is also possible that different heating rates lead to diverse availability of oxygen, thus affecting lipid oxidation in a different manner due to the action of antioxidants as phenolic compounds. In any case, the chemical pathway of the thermo-degradative reactions of these antioxidant compounds needs further investigation, as well as the formation, chemical structure and behavior of oxidized phenols [1].

Conclusion

In light of the results obtained in this study, it was possible to conclude that such parameters allow the investigation of the microwave heating of four Tunisian olive oils. This treatment produces significant losses in olive oil quality. In addition, the extension of losses is higher when the time of exposure increases. With the exception of free acidity values, all parameters are significantly affected by the time of heating. In what concern to pigments such chlorophylls and carotenoids content, we can conclude that they are thermal labile, once that their quantity decrease as long as the exposure time increase. Based on these scientific findings, the use of

microwave is not discouraged, but olive oils heating should be reduced to the minimum, in order to reduce the degradation extent of important compounds, as chlorophyll, carotenoid pigments, and phenolics, while reducing the formation of potentially hazard components, the oxidized lipids.

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