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SARI, Hafize Ayla; EKINCI, Raci

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The effect of ultrasound application and addition of leaves in the malaxation of olive oil extraction on the olive oil yield, oxidative stability and organoleptic quality

Hafize Ayla SARI[1]*, Raci EKINCI[2]

Abstract

This research examines the effect of adding olive leaf on the yield and quality of the olive oil extracted from malaxation and ultrasound application during the olive oil extraction process. The olive variety Ayvalık was used in the trials, which included leaf addition of 2% and 5% and ultrasound application of 5, 10, 15, 20, 25, 30, 35, and 40 minutes (min). Several values, such as maturity index and oil yield, as well as olive oil parameters, including free acidity, peroxide value, oxidative stability, and specific ultraviolet absorption, were analyzed and examined. We also carried out sensory analyses on the extracted olive oils. The results show that the product extracted has no sensory defects and exhibits a fruitiness level greater than 0. The research showed that, in terms of yield and quality, a time span of 15 min with addition of 2% olive leaf would provide optimum conditions in ultrasound assisted olive oil extraction with olive leaf addition. The olive oils produced in this process fall into the category of "extra virgin olive oil".

Keywords: olive oil; olive leaves; ultrasound; olive oil quality; sensory analysis.

Practical Application: Ultrasound application and leaf addition can be used to increase oil yield and sensory properties.

1 Introduction

The olive tree is found in diverse habitats in certain regions in the world, but the Mediterranean basin is the usual habitat of olive tree, and the species has become naturalized in this favorable ecological environment (Cronquist, 1981).

It is reported that 88 varieties of olive grow in Turkey. Memecik, the most common, makes up 74% of the olive tree population and is used for oil extraction. The Ayvalık variety ranks second behind Memecik (Gümüşkesen & Yemişcioğlu, 2007), and it makes up 19% of the total olive tree population in Turkey and 25% of those growing in the Aegean region (Kayahan & Tekin, 2009; Efe et al., 2011; Özkaya et al., 2009).

The Ayvalık olive fruit, also called Edremit, ripens early and is semi-resistant to cold. It generally used for oil extraction. Although it yields a fruit with a large stone and rough surface, which is non-uniform in size, it is consumed as green, pink and black table olives due to its high oil rate and intense aroma. The pulp/stone ratio of the Ayvalık variety is 4-5/1, and its oil rate amounts to 24-26%. There are 260-300 olives in one kg (Gümüşkesen et al., 2003).

The positive effects on human health and the sensory properties of olive oil have led to increasing demand in recent years (Forina et al., 2007). In terms of the food industry, the production of high quality extra virgin olive oil with a rich content of aromatic compounds is a process of great significance. It is a known fact that the method and conditions of oil extraction from olive also have an effect on the quality and aroma of the olive oil produced. Diverse processing conditions, especially different malaxation temperatures and times, can change the content of volatile compounds in the oil (Angerosa et al., 2001; Di Giovacchino et al., 2002; Kalua et al., 2006; Ranalli et al., 2001; Ranalli et al., 2003). From a sensory perspective, olive oil should have a fruity aroma that adequately combines all the aspects of the sort or sorts from which it has been extracted (Vossen, 2007).

Olive leaves, whose characteristics make them a highly valuable biological material, can be a healthy, reliable, cheap, effective, and alternative source with antioxidant properties, and they help extend the shelf life of foods, preventing losses to their sensory and nutritional properties (Jemai et al., 2009; Boudhrioua et al., 2009; Bouaziz et al., 2010).

Some methods of heat processing used in processing and conservation of foods lead to vitamin loss, enzyme inactivation and spoilage of natural compounds. For such reasons, foods are treated with other non-thermal processes, such as irradiation, pulsed electric field, ultrasound application and ultraviolet (UV) light (Yüksel, 2013).

Ultrasound technology is based on ultrasonic sound waves, which are generated at a frequency threshold that cannot be heard by humans (Ulusoy & Karakaya, 2011). Ultrasound assisted extraction is a non-thermal alternative method. The ultrasound technique is used for protein and oil extraction. Ultrasonic application mechanically disrupts the cell walls and facilitates the extraction process. It leads to a reduction of oil in the pomace (Bayraktaroğlu & Obuz, 2006).

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[1] *Food Technology Department, Çine Vocational School, Adnan Menderes University, Suleyman Pekguzel Campus, Çine, Aydın-Turkey*
[2] *Food Engineering Department, Faculty of Engineering, Pamukkale University, Kınıklı Campus, Denizli, Turkey*
*Corresponding author: asari@adu.edu.tr

In 2008, Luque de Castro and collaborators developed the first direct enrichment of olive oil using olive leaves in a continuous extraction assisted by ultrasound (Japon-Lujan et al., 2008).

This research aims to specify the effects of ultrasound application and addition of leaves during the malaxation process on the yield amount, oxidative stability, and sensory properties of the oil.

2 Materials and Methods

2.1 Samples and chemicals

Olives of the Ayvalık variety were used in this research and were harvested from a grove in the district of Çine, in the province of Aydın, in the harvest years of 2014-2015 and 2015-2016. All the olives were harvested in the early harvest period (pink stage), during which the skin of the olives was yellow or greenish yellow. Fresh leaves picked from the tops of the trees were dried at 50 °C in a tray drier and cut up into 5 mm pieces. The leaves were subjected to a drying process within at most 24 hours after they were picked. The extracted olive oil was stored in black colored glass containers at 4 °C until analyzed.

All reagents used were analytical or HPLC grade. Hexane was obtained from Sigma Aldrich Co. (catalogue no: 208752, Sigma-AldrichChemie, Steinheim). Potassium iodide (catalogue no: 105043), diethyl ether (catalogue no: 100921), acetic acid (catalogue no: 815035), chloroform (catalogue no: 102445), phenolphthalein (catalogue no: 1.07233.0100), and sodium hydroxide (catalogue no: 106462) were obtained from Merck Millipore (Darmstadt, Germany).

2.2 Instrumentation

Ultrasound-assisted maceration

Leaves were added at 2% or 5% per 3500 g olives for each extraction process. In the malaxation stage, after crushing the olives in an Abencor system (MC2 Ingenieria Sistemas, Seville, Spain) with a hammer crusher, the mixture was subjected to a kneading process totaling 40 min; in parallel, an ultrasound application was conducted for 0, 5, 10, 15, 20, 25, 30, 35, and 40 min at a 26 kHz ultrasonic frequency and at 200 W with a titanium probe ultrasound device (Hielscher UP200, Germany) in a 3 L tank volume. The solid and liquid phases were separated through a hydro-extraction at 5000 rpm. Then, the olive oil was decomposed through natural decantation from the vegetable water and purified by filtering.

3 Methods

3.1 Determination maturity of index

The maturity index was determined for 100 randomly selected olives. Each sample was selected and classified according to one of the seven levels of maturity as defined by Boskou (1996), from 0 (olive skin intense green color) to 7.

3.2 Determination of peroxide value

The peroxide value was determined in accordance with 1989 AOCS Official Method Cd 8-53 (American Oil Chemists Society, 1989a). Thus, 50 mL of acetic acid:chloroform (3:2) and a saturated KI solution was injected into 5 g of oil by means of a 0.5 mL syringe and then slowly shaken by hand for 1 min. After mixing with 30 mL distilled water and 1 mL of a starch solution indicator at 5 g/1000 mL, it was subjected to a titration with 0.1 N $Na_2S_2O_3$ until it regained its initial color.

3.3 Determination of oxidative stability

Thermal oxidative stability tests of olive oil samples were carried out in accordance with the drying oven method identified by Skevin et al. (2003). A 40 mL olive oil sample was put in a Petri plate and was subjected to oxidation in a drying oven with a light inside at 65 (±3) °C for 7 days. Thermal oxidative stability values of the sample were calculated by means of the formula indicated below based on the change in peroxide number values (Equation 1).

$$\textit{Change in peroxide number}\ \% = PS2 - PS1 / PS1 \times 100 \quad (1)$$

PS1: Initial calculated peroxide number

PS2: Calculated peroxide number after a 7 day heat treatment

3.4 Determination of UV absorption

Determination of UV absorption (K_{232} and K_{270}) was carried out according to the analytical methods described in AOCS Official Method Ch 5-91 (American Oil Chemists Society, 1989b). Briefly, a 0.25 g sample, sieved in filter paper, was dissolved with 25 mL cyclohexane in a volumetric flask. Measurements were carried out in a spectrophotometer using a 1 cm quartz cuvette at 232 and 270 nm compared with cyclohexane using rapid movements.

3.5 Sensory evolution

The analysis of the extracted olive oils was carried out in accordance with the "COI/T.20/Doc.15/Rev.6-2013 Organoleptic Assessment of Virgin Olive Oil" method (International Olive Oil Council, 2013). In this method, a trained panel of 8-12 individuals defines and classifies the rates of fruitiness, bitterness, pungency, and defects of the oil. The panellists, led by the tasting supervisor, assessed the positive and negative attributes they found on a scale of 10 cm using appropriate tasting glasses in a room with controlled conditions. While positive attributes are fruitiness, bitterness, and pungency, parameters such as heated-muddy residue, musty-moist-earthy, winey-vinegary-acidic, wet-woody, rancid-stale, metallic, and black water are assessed as negative attributes (Escuderos et al., 2007).

3.6 Further measurements

Free fatty acid content was determined according to AOCS Official Method Ca 5a-40. (American Oil Chemists Society, 1989c) Extraction yields were calculated according to the formula indicated below, with the mean value for 3 repetitions attained for each ultrasound application (Equation 2).

$$\textit{Extraction yields} = \textit{extracted oil rate} / \textit{total oil rate}\ (\%) \times 100 \quad (2)$$

3.7 Statistical analysis

Statistical analysis of the data was carried out by analysis of variance (ANOVA) and Tukey's multiple range test to show measurements that could be considered significantly different. A significance level of $p < 0.05$ was used. All statistical analyses were performed using the SPSS statistics software package (version 16.0; IBM Corporation, NY, USA).

4 Results and discussion

Olive oil was extracted from the Ayvalık variety with and without adding leaves. Leaves were added at 2% and 5%, subjecting them to the ultrasound process for different lengths of time or to malaxation for a 40 min. Temperature changes that occurred in the kneading stage are shown in Table 1.

While the temperature before malaxation was 20.7 °C in studies carried out in 2014-2015, in this study, we observed a temperature difference of 26.8 °C, i.e., between 22.7 and 49.5 °C, in the olive paste kneaded via the ultrasound assisted process, depending on the ultrasound application. The initial temperature in 2015-2016 was 20.5 °C; however, the temperatures varied between 23.3 and 48.7 °C after the application, resulting in a maximum temperature difference of 25.4 °C. The rates of temperature increase in these two periods showed a similar trend. This temperature increase was generated both by the ultrasonic thermal effect and the malaxation process.

The oil extraction yields for the ultrasound-assisted process for different lengths of time after crushing the olives and processed without or with the addition of 2% or 5% leaves is shown in Table 2.

The maximum extraction yield was attained with 2% leaf addition and ultrasound application of 15 min, with values of 50% for 2014-2015 and 46% for 2015-2016. The study showed that the yields attained from control samples processed without adding leaves were higher than those attained from samples treated with leaf addition of 5%. Extraction yield for the process with ultrasound application showed a parallel tendency, i.e., first an upward, then a downward trend, in the two harvest years. While the process with leaf addition of 2% and 15 min of ultrasonic application gave the maximum extraction output, the lowest output values were attained in the process that used a leaf addition of 5%.

Even though an increase in temperature enables oil extraction and consequently leads to an increase in the yield, an emulsion formed during long ultrasound application times

Table 1. Temperature changes of olive oil without adding leaf and by adding leaves in the rate of 2% and 5% subjecting them to ultrasound process.

Leaves (%)	Sonication time (min)	Malaxation time (min)	Inlet temperature of ultrasound (°C)	Outlet temperature of ultrasound (°C)	Inlet temperature of ultrasound (°C)	Outlet temperature of ultrasound (°C)
			2014-2015		2015-2016	
Control	0	40	20.7	22.7	20.5	23.3
	5	35	20.7	23.5	20.5	24.2
	10	30	20.7	24.7	20.5	25.7
	15	25	20.7	25.3	20.5	26.4
	20	20	20.7	32.9	20.5	31.5
	25	15	20.7	35.3	20.5	36.7
	30	10	20.7	39.2	20.5	38.2
	35	5	20.7	41.5	20.5	40.5
	40	0	20.7	48.7	20.5	46.3
2%	0	40	20.7	22.7	20.5	23.3
	5	35	20.7	22.9	20.5	23.9
	10	30	20.7	24.8	20.5	25.7
	15	25	20.7	24.9	20.5	25.4
	20	20	20.7	26.2	20.5	27.4
	25	15	20.7	34.4	20.5	32.5
	30	10	20.7	35.8	20.5	36.8
	35	5	20.7	40.2	20.5	39.4
	40	0	20.7	42.5	20.5	41.4
5%	0	40	20.7	22.7	20.5	23.3
	5	35	20.7	24.7	20.5	26.6
	10	30	20.7	25.3	20.5	26.4
	15	25	20.7	26.3	20.5	28.6
	20	20	20.7	33.7	20.5	33.5
	25	15	20.7	34.3	20.5	36.9
	30	10	20.7	40.4	20.5	40.3
	35	5	20.7	43.5	20.5	42.5
	40	0	20.7	49.5	20.5	48.7

Table 2. Yield efficiency and oil extractability of olive oil without adding leaf and by adding leaves in the rate of 2% and 5% subjecting them to ultrasound process.

Leaves (%)	Sonication time (min)	Yield efficiency (%)		Oil extractability (%)	
		2014-2015	2015-2016	2014-2015	2015-2016
Control	0	$11^{b} \pm 1$	$11^{b} \pm 1$	$45^{d} \pm 5$	$43^{d} \pm 4$
	5	$11^{b} \pm 1$	$11^{b} \pm 1$	$45^{d} \pm 4$	$44^{c} \pm 6$
	10	$11^{b} \pm 1$	$11^{b} \pm 1$	$45^{cd} \pm 6$	$44^{bc} \pm 5$
	15	$11^{b} \pm 1$	$11^{ab} \pm 1$	$46^{c} \pm 6$	$45^{a} \pm 4$
	20	$11^{b} \pm 1$	$11^{ab} \pm 1$	$45^{c} \pm 5$	$45^{b} \pm 5$
	25	$11^{b} \pm 1$	$11^{b} \pm 1$	$45^{d} \pm 4$	$45^{b} \pm 5$
	30	$10^{bc} \pm 1$	$10^{b} \pm 1$	$43^{e} \pm 4$	$42^{d} \pm 4$
	35	$10^{c} \pm 1$	$10^{c} \pm 1$	$42^{e} \pm 6$	$41^{ef} \pm 5$
	40	$10^{c} \pm 1$	$10^{c} \pm 1$	$41^{f} \pm 4$	$40^{f} \pm 5$
2%	0	$10^{b} \pm 1$	$11^{b} \pm 1$	$45^{cd} \pm 5$	$43^{cd} \pm 5$
	5	$11^{b} \pm 1$	$11^{ab} \pm 1$	$48^{b} \pm 4$	$44^{c} \pm 5$
	10	$11^{b} \pm 1$	$11^{a} \pm 1$	$48^{b} \pm 5$	$45^{b} \pm 5$
	15	$11^{a} \pm 2$	$12^{a} \pm 1$	$50^{a} \pm 7$	$46^{a} \pm 5$
	20	$11^{ab} \pm 1$	$11^{a} \pm 1$	$50^{a} \pm 6$	$45^{a} \pm 5$
	25	$11^{ab} \pm 1$	$11^{a} \pm 1$	$49^{a} \pm 6$	$45^{ab} \pm 5$
	30	$11^{b} \pm 1$	$11^{ab} \pm 1$	$47^{b} \pm 5$	$44^{c} \pm 5$
	35	$10^{c} \pm 1$	$11^{b} \pm 1$	$44^{e} \pm 4$	$41^{e} \pm 5$
	40	$10^{c} \pm 1$	$10^{b} \pm 1$	$43^{e} \pm 5$	$41^{ef} \pm 5$
5%	0	$11^{ab} \pm 1$	$11^{b} \pm 1$	$44^{e} \pm 4$	$42^{e} \pm 4$
	5	$11^{ab} \pm 1$	$11^{a} \pm 1$	$44^{de} \pm 5$	$43^{d} \pm 4$
	10	$11^{ab} \pm 1$	$11^{a} \pm 1$	$44^{d} \pm 5$	$44^{c} \pm 4$
	15	$11^{a} \pm 1$	$12^{a} \pm 1$	$45^{d} \pm 4$	$45^{a} \pm 6$
	20	$11^{ab} \pm 1$	$12^{a} \pm 1$	$44^{d} \pm 4$	$44^{bc} \pm 4$
	25	$11^{b} \pm 1$	$12^{a} \pm 1$	$42^{e} \pm 5$	$44^{c} \pm 5$
	30	$11^{b} \pm 1$	$11^{ab} \pm 1$	$41^{f} \pm 4$	$43^{d} \pm 5$
	35	$11^{b} \pm 1$	$11^{b} \pm 1$	$41^{f} \pm 6$	$40^{f} \pm 5$
	40	$10^{b} \pm 1$	$10^{b} \pm 1$	$41^{g} \pm 4$	$39^{fg} \pm 5$

Values are the mean ± Standard deviation (n = 3). Means with the same letter in the same column are not significantly different at $p < 0.05$.

in the malaxation stage, making it difficult to separate the oil from the vegetable water and therefore preventing a complete phase separation. We hypothesize that application times gave rise to a reduction in the oil yield.

Moreover, it was expected that leaf addition would not only pass through phenolic and aromatic compounds to the oil but also enable the transfer of enzymes to the extracted product. In the application in which leaves were added into the paste at 2%, these enzymes enabled the highest yield. However, for the 5% leaf addition, different emulsions formed, resulting in a reduced quantity of oil extracted because more enzymes were transferred in this application than were desired.

Table 3 shows the free acidity, peroxide value, specific absorption value under UV and oxidative stability of the oil extracted using the ultrasound-assisted process for different lengths of time after crushing the olives and with or without adding leaves at 2% and 5%.

The free fatty acid content of the olive oils extracted with leaf addition and the ultrasonic process in the 2014-2015 and 2015-2016 harvest years varied between 0.32-0.40 and 0.41-0.50, respectively. While ultrasound application led to an increase in free acidity in both periods, the values attained in 2015-2016 were higher than the free acidity attained in 2014-2015. It is thought that this increase is due to the climate conditions. Furthermore, the study showed that free acidity values attained from ultrasound application where leaves were added at 5% were higher than those attained in the control process or with 2% leaf addition. The results of this research support our assumption that the vanillic, caffeic and elenolic acids in olive leaves give rise to an increase in the free acidity of olive oils extracted using the ultrasound assisted process compared with no leaf addition for the same period and the same ultrasound treatment time.

Peroxide values, expressed in meqO_2/kg, of the oils extracted in the 2014-2015 and 2015-2016 harvest years varied between 6.4-11.6 and 8.6-13.1, respectively. The highest peroxide value attained in both periods was recorded for the process with an ultrasound application of 40 min, 5% leaf addition and malaxation. The lowest value attained in both periods, on the other hand, was observed in the process with ultrasound application of 15 min and leaf addition of 2%. The research showed that the results concerning the peroxide values in both of the periods involved were within the limits (≤20) defined in the European Commission Regulation 1989/2003 for peroxide values of olive oils.

Table 3. Some chemical and quality properties of olive oil without adding leaf and by adding leaves in the rate of 2% and 5% subjecting them to ultrasound process.

Leaves (%)	Sonication time(min.)	Free acidity (% Oleic acid)		Peroxide value (meq O_2/kg)		K_{232}		K_{270}		Oxidative stability	
		2014-2015	2015-2016	2014-2015	2015-2016	2014-2015	2015-2016	2014-2015	2015-2016	2014-2015	2015-2016
Control	0	$0.32^{g} \pm 0.04$	$0.41^{g} \pm 0.02$	$8.4^{c} \pm 0.3$	$10.2^{bc} \pm 0.1$	$1.9^{a} \pm 0.1$	$2.0^{a} \pm 0$	$0.14^{a} \pm 0.02$	$0.14^{a} \pm 0.02$	$95^{de} \pm 8$	$90^{de} \pm 8$
	5	$0.34^{f} \pm 0.05$	$0.43^{f} \pm 0.01$	$8.2^{c} \pm 0.1$	$10.0^{c} \pm 0.2$	$1.8^{c} \pm 0$	$1.8^{b} \pm 0$	$0.13^{b} \pm 0.01$	$0.13^{b} \pm 0.01$	$105^{d} \pm 11$	$99^{d} \pm 12$
	10	$0.35^{fe} \pm 0.03$	$0.43^{ef} \pm 0.02$	$7.9^{cd} \pm 0.3$	$9.9^{c} \pm 0.1$	$1.6^{cd} \pm 0$	$1.7^{b} \pm 0$	$0.13^{b} \pm 0.001$	$0.13^{b} \pm 0.01$	$119^{d} \pm 15$	$101^{d} \pm 8$
	15	$0.35^{e} \pm 0.02$	$0.44^{e} \pm 0.03$	$7.8^{cd} \pm 0.1$	$8.7^{d} \pm 0.1$	$1.5^{e} \pm 0$	$1.6^{c} \pm 0$	$0.09^{e} \pm 0.001$	$0.11^{d} \pm 0.01$	$141^{b} \pm 9$	$124^{c} \pm 7$
	20	$0.36^{d} \pm 0.01$	$0.45^{de} \pm 0.04$	$8.2^{c} \pm 0.2$	$9.9^{c} \pm 0.2$	$1.6^{d} \pm 0.1$	$1.6^{bc} \pm 0.1$	$0.11^{d} \pm 0.01$	$0.12^{c} \pm 0.02$	$128^{cd} \pm 16$	$116^{d} \pm 6$
	25	$0.36^{d} \pm 0.05$	$0.46^{d} \pm 0.02$	$8.3^{c} \pm 0.1$	$9.8^{c} \pm 0.1$	$1.7^{c} \pm 0.1$	$1.7^{b} \pm 0.3$	$0.12^{c} \pm 0.001$	$0.13^{b} \pm 0.01$	$111^{d} \pm 7$	$110^{d} \pm 8$
	30	$0.37^{dc} \pm 0.03$	$0.46^{d} \pm 0.01$	$8.3^{c} \pm 0.1$	$10.3^{b} \pm 0.2$	$1.8^{b} \pm 0.2$	$1.6^{c} \pm 0$	$0.13^{b} \pm 0.02$	$0.13^{b} \pm 0.03$	$107^{d} \pm 6$	$105^{d} \pm 6$
	35	$0.37^{dc} \pm 0.01$	$0.46^{d} \pm 0.02$	$8.8^{c} \pm 0.1$	$11.6^{a} \pm 0.1$	$2.0^{a} \pm 0$	$1.8^{b} \pm 0$	$0.14^{a} \pm 0.02$	$0.15^{a} \pm 0.03$	$106^{d} \pm 11$	$101^{d} \pm 10$
	40	$0.37^{d} \pm 0.03$	$0.47^{c} \pm 0.03$	$9.5^{b} \pm 0.1$	$12.3^{a} \pm 0.2$	$2.1^{a} \pm 0$	$1.7^{b} \pm 0$	$0.15^{a} \pm 0.001$	$0.16^{a} \pm 0.01$	$99^{de} \pm 5$	$98^{d} \pm 7$
2%	0	$0.35^{f} \pm 0.01$	$0.43^{ef} \pm 0.02$	$7.7^{cd} \pm 0.1$	$9.8^{c} \pm 0.2$	$1.8^{c} \pm 0$	$1.9^{a} \pm 0$	$0.12^{c} \pm 0.001$	$0.13^{b} \pm 0.01$	$110^{d} \pm 9$	$102^{d} \pm 12$
	5	$0.35^{e} \pm 0.03$	$0.44^{e} \pm 0.03$	$7.1^{d} \pm 0.3$	$9.6^{c} \pm 0.1$	$1.6^{d} \pm 0$	$1.6^{bc} \pm 0$	$0.11^{d} \pm 0.02$	$0.11^{d} \pm 0.02$	$120^{cd} \pm 8$	$129^{c} \pm 9$
	10	$0.36^{de} \pm 0.02$	$0.44^{e} \pm 0.01$	$6.9^{d} \pm 0.1$	$9.4^{c} \pm 0.2$	$1.5^{d} \pm 0$	$1.5^{c} \pm 0$	$0.12^{c} \pm 0.02$	$0.12^{c} \pm 0.02$	$140^{bc} \pm 7$	$122^{c} \pm 9$
	15	$0.36^{d} \pm 0.02$	$0.46^{de} \pm 0.05$	$6.4^{d} \pm 0.1$	$8.6^{d} \pm 0.1$	$1.4^{d} \pm 0$	$1.3^{e} \pm 0$	$0.07^{f} \pm 0.01$	$0.09^{f} \pm 0.03$	$152^{a} \pm 3$	$153^{a} \pm 8$
	20	$0.37^{c} \pm 0.04$	$0.46^{d} \pm 0.04$	$7.1^{d} \pm 0.3$	$9.8^{c} \pm 0.1$	$1.4^{d} \pm 0$	$1.4^{d} \pm 0$	$0.09^{e} \pm 0.02$	$0.11^{d} \pm 0.02$	$149^{ab} \pm 8$	$144^{b} \pm 8$
	25	$0.38^{c} \pm 0.01$	$0.47^{d} \pm 0.02$	$7.3^{d} \pm 0.1$	$9.8^{c} \pm 0.1$	$1.7^{c} \pm 0$	$1.5^{c} \pm 0$	$0.11^{d} \pm 0.02$	$0.12^{c} \pm 0$	$149^{ab} \pm 15$	$141^{b} \pm 9$
	30	$0.38^{b} \pm 0.03$	$0.47^{c} \pm 0.04$	$7.6^{cd} \pm 0.3$	$9.9^{c} \pm 0.2$	$1.8^{c} \pm 0$	$1.5^{d} \pm 0$	$0.11^{d} \pm 0.01$	$0.10^{e} \pm 0.02$	$145^{b} \pm 9$	$127^{c} \pm 8$
	35	$0.39^{b} \pm 0.04$	$0.48^{b} \pm 0.0.2$	$7.7^{cd} \pm 0.1$	$10.8^{b} \pm 0.2$	$1.9^{b} \pm 0$	$1.7^{b} \pm 0$	$0.12^{c} \pm 0.02$	$0.13^{b} \pm 0.01$	$117^{d} \pm 15$	$130^{c} \pm 8$
	40	$0.39^{a} \pm 0.02$	$0.49^{b} \pm 0.01$	$10.2^{a} \pm 0.4$	$11.7^{a} \pm 0.2$	$1.9^{a} \pm 0$	$1.7^{b} \pm 0$	$0.13^{b} \pm 0.01$	$0.13^{b} \pm 0.02$	$103^{d} \pm 16$	$106^{d} \pm 6$
5%	0	$0.36^{d} \pm 0.03$	$0.45^{de} \pm 0.03$	$10.1^{a} \pm 0.1$	$11.4^{a} \pm 0.1$	$1.9^{b} \pm 0$	$1.8^{a} \pm 0$	$0.14^{a} \pm 0.01$	$0.14^{a} \pm 0.01$	$124^{cd} \pm 10$	$121^{c} \pm 12$
	5	$0.37^{d} \pm 0.04$	$0.47^{d} \pm 0.02$	$9.8^{ab} \pm 0.2$	$11.5^{a} \pm 0.2$	$1.7^{c} \pm 0$	$1.6^{c} \pm 0$	$0.12^{c} \pm 0.01$	$0.13^{b} \pm 0.02$	$135^{c} \pm 16$	$142^{b} \pm 17$
	10	$0.38^{c} \pm 0.01$	$0.47^{d} \pm 0.04$	$8.7^{c} \pm 0.2$	$10.7^{b} \pm 0.5$	$1.6^{d} \pm 0$	$1.4^{d} \pm 0$	$0.13^{b} \pm 0.01$	$0.13^{b} \pm 0.02$	$147^{b} \pm 11$	$153^{a} \pm 12$
	15	$0.38^{b} \pm 0.03$	$0.47^{c} \pm 0.01$	$8.4^{c} \pm 0.1$	$10.3^{b} \pm 0.2$	$1.4^{d} \pm 0$	$1.1^{e} \pm 0$	$0.09^{e} \pm 0.01$	$0.11^{d} \pm 0$	$161^{a} \pm 9$	$160^{a} \pm 5$
	20	$0.39^{b} \pm 0.03$	$0.47^{c} \pm 0.02$	$8.8^{c} \pm 0.2$	$10.8^{b} \pm 0.2$	$1.5^{d} \pm 0$	$1.4^{d} \pm 0$	$0.12^{c} \pm 0.02$	$0.13^{b} \pm 0.01$	$155^{a} \pm 7$	$155^{a} \pm 7$
	25	$0.39^{a} \pm 0.02$	$0.48^{c} \pm 0.02$	$9.2^{b} \pm 0.1$	$10.9^{b} \pm 0.1$	$1.7^{c} \pm 0$	$1.5^{d} \pm 0$	$0.12^{c} \pm 0.01$	$0.14^{a} \pm 0.02$	$150^{a} \pm 10$	$145^{b} \pm 12$
	30	$0.39^{a} \pm 0.01$	$0.48^{b} \pm 0.03$	$9.4^{b} \pm 0.1$	$11.6^{a} \pm 0.1$	$1.9^{b} \pm 0$	$1.5^{c} \pm 0$	$0.12c \pm 0.02$	$0.11^{d} \pm 0.02$	$144^{b} \pm 4$	$142^{b} \pm 11$
	35	$0.40^{a} \pm 0.02$	$0.49^{b} \pm 0.02$	$10.3^{a} \pm 0.3$	$12.8^{a} \pm 0.2$	$1.9^{a} \pm 0$	$1.6^{c} \pm 0$	$0.12^{c} \pm 0.03$	$0.14^{a} \pm 0.01$	$132^{c} \pm 13$	$139^{b} \pm 11$
	40	$0.40^{a} \pm 0.03$	$0.50^{a} \pm 0.01$	$11.6^{a} \pm 0.1$	$13.1^{a} \pm 0.1$	$2.0^{a} \pm 0$	$1.6^{c} \pm 0$	$0.14^{a} \pm 0.01$	$0.15^{a} \pm 0.02$	$121^{cd} \pm 12$	$121^{c} \pm 12$

Values are the mean ± Standard deviation (n = 3). Means with the same letter in the same column are not significantly different at $p < 0.05$.

While the K_{232} values regarding the absorption values of the olive oil samples extracted in the 2014-2015 and 2015-2016 harvest years under UV were between 1.4-2.0 and 1.3-2.0, respectively, the K_{270} values varied between 0.07-0.15 and 0.09-0.16, respectively. The longer contact time with oil and oxygen in malaxation stage may cause and increase in conjugated structures. However, it is thought that K_{232} and K_{270} values rise in olive oils exposed to heat.

The values for oxidative stability of the oils extracted in the 2014-2015 and 2015-2016 harvest years varied between 95-161 and 90-160, respectively. It is thought that the increase in peroxide numbers is generated by an increase in peroxidase and polyphenol oxidase enzymes that occurs in parallel with the increase in ultrasound time and temperature along with the kneading in the malaxation stage. The increase in oxidative stability arises from the oxidative reaction of these enzymes with phenolic compounds in the presence of oxygen and by releasing the phenolics, especially at places near the cell membrane, along with substantial tissue fractionation.

Based on the results of this study, olive leaf extract is a good source of polyphenols, mainly oleuropein, hydroxytyrosol, and quercetin. Addition of this type of extract to edible olive oils may increase the radical scavenging activity and oxidative stability of olive oils.

Figure 1 shows the results of the sensory analysis for the 2014-2015 harvest year regarding the oil extracted with the ultrasound assisted process applied for different times after crushing the olives and without and with the addition of 2% or 5%. The results for 2015-2016 are shown in Figure 2.

There was no difference in the sensory analysis of the olive oils between harvest years in terms of fruitiness, bitterness and pungency intensities; the panellists reached very similar evaluation results, which showed no statistically significant change. The average of the fruitiness intensity of the oils extracted in the 2014-2015 and 2015-2016 harvest years varied between 1.00-3.54/1.02-3.50, that of pungency between 1.35-1.65/1.30-1.72 and that of bitterness, on the other hand, between 1.00-1.50/1.05-1.49. The analysis did not indicate any defects, and the fruitiness intensity was found to be greater than 0. The research showed, with regards to inter-relation between sensory properties, a statistically significant positive correlation between the positive property of fruitiness and the properties of pungency and bitterness. Likewise, a statistically significant

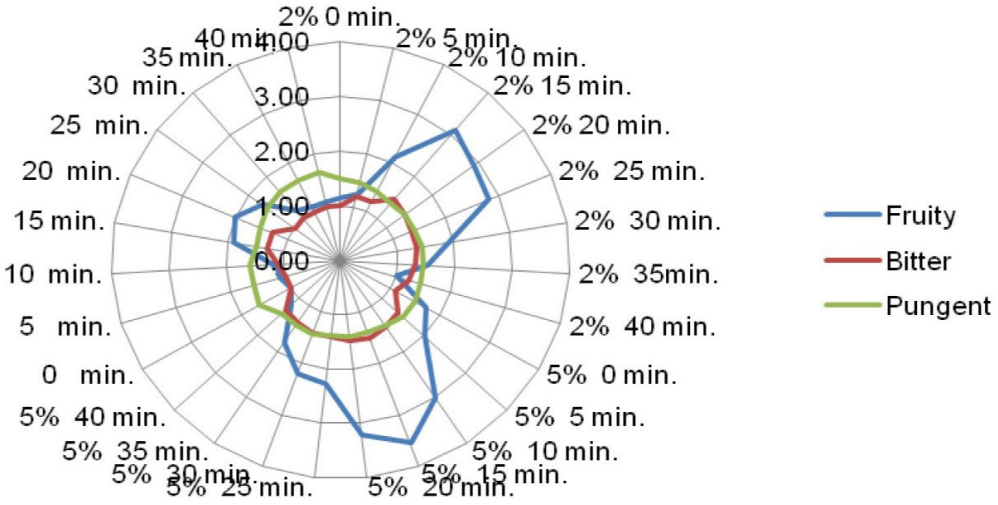

Figure 1. Sensory analysis results of the 2014-2015 year.

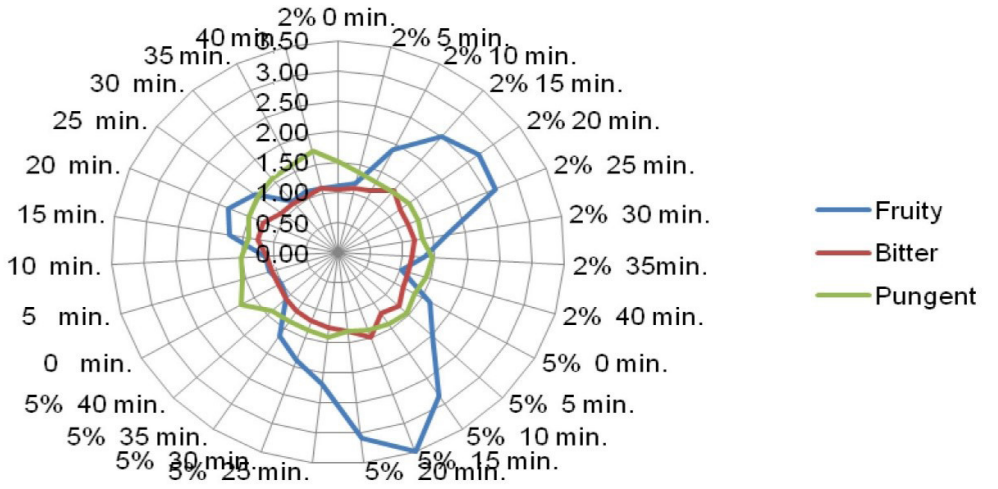

Figure 2. Sensory analysis results of the 2015-2016 year.

strong positive relationship was determined between pungency and bitterness.

5 Conclusion

The results of this research show that leaf addition and ultrasound application have a significant effect on the yield, quality criteria, and sensory properties of olive oil. Additionally, it was found that leaf addition of 2% and an ultrasound application for 15 min in the malaxation stage provided the optimum conditions to extract olive oil with the highest yield and lowest free acidity. This research was carried out with the aim to achieve maximum product quality and yield; the malaxation process performed under the experimental conditions with leaf addition and ultrasound application gave positive results. It can be concluded that ultrasound application enables an olive oil extraction in a shorter time, with better quality and at lower costs compared with the same quantity of product extracted by traditional methods in industry.

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