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Use of Enzymatic Preparations to Improve the Productivity and Quality of Olive Oil

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ABSTRACT

Improving the quantity and quality of olive oil extraction is considered crucial for producers not only in Jordan but also worldwide. The present study aimed to enhance olive oil yield from olive fruits without compromising its quality by applying an enzymatic treatment technique during the malaxation stage of the oil extraction process. The enzymes that were used in this study were cellulase, pectinase, and a mixture of both enzymes at a ratio of (1:1). Each of the enzymes and there mixture was added at concentrations of 0.02 %, 0.04 % 0.06 %, 0.08 %, 0.10 %, and 0.12 % (w/w). Two Jordanian olive cultivars, Nabali Baladi (NB) and Nabali Muhassan (NM), in their immature state, were selected for the enzymatic treatment. The olive oil yield increased significantly ($P < 0.05$) after the enzymatic treatment. The increments in yield were 4.38 % (at an enzymatic concentration of 0.08 %), 3.29 % (at 0.1 %), and 5.25% (at 0.12 %) for NB treated with cellulase, pectinase, and 1:1 cellulase/pectinase, respectively. The increments in oil yield were 4.08 % (at 0.1%), 3.09 % (at 0.12%), and 4.5 (at 0.08%) for NM treated with cellulase, pectinase, and 1:1 cellulase/pectinase, respectively. The percent increments were significantly ($P < 0.05$) higher for NB than for NM. The quality parameters in terms of acidity, peroxide values, and UV-extinction coefficients at 232 and 270 nm were not significantly affected in any of the treatment groups when compared to those of control samples. The content of phenolic compounds, α-tocopherols, chlorophylls, and carotene was significantly higher ($P < 0.05$) in both oils in all enzymatic treatments than in the control, resulting in increased oxidative stability, as revealed by Rancimat analyses.

Keywords: Olive oil extractability; Enzymatic treatment; Standard olive oil quality; Antioxidant compounds.

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INTRODUCTION

Olive oil (*Olea europaea* L.) has been an essential food source for the Mediterranean region for thousands of years, providing a wealth of health-promoting constituents, mainly antioxidant agents such as tocopherols, phenolic compounds, chlorophyll, and carotenoids, in addition to a high level of monounsaturated fatty acids (Najafian *et al*., 2009; Servili *et al*., 2014; Vitagloine *et al*., 2015). Moreover, olive oil is widely used in many industries such as pharmaceuticals (alopecia, paralysis, rheumatic pain, and hypertension), cosmetics, soap manufacture, textile, and lubricants (Rakesh and Sharma, 2007; Waterman *et al.,* 2007). Considering the above-mentioned reasons, global awareness has developed regarding the importance of olive oil, which has led to an increase in the demand for it. Therefore, it is necessary to develop new technologies to increase olive oil production without sacrificing its quality.

Through the ages, olive oil extraction has been performed using several methods, including mechanical, chemical, and physical methods or a combination of these. The ideal objective of any extraction method is to extract the largest possible amount of oil without altering its original quality (Dounia *et al.,* 2014; Favati, 2017; Thaiza *et al.,* 2020).

In all extraction processes, the major concern is the quantity of oil obtained (~ 24%) as the oil remains trapped inside the colloidal suspension in the cytoplasm or is emulsified with vegetative water, and thus is not recoverable or is lost with the extraction byproducts (Rakesh and Sharma, 2007). Reduction in the amount of oil lost during the extraction process requires degradation of the cell membranes of the olive fruit. The plant cell wall is composed mainly of cellulose, pectin, hemicelluloses, glycoproteins, and lignin, which maintain cell rigidity. The endogenous cellular enzyme activities lead to softening of the cell walls during ripening. These enzymes are significantly inactivated during the oil extraction process (Ranalli and De Mattia 1997; Najafian *et al*., 2009). This requires using enzymes from outside sources to compensate for the absence of natural enzymes. Many researchers suggest adding specific commercial enzymes at the beginning of the malaxation stage of forming an olive paste. The breakdown of the cell walls in olive fruit tissue cells, emulsions, and colloidal solutions that form in the vegetative water of olive paste can alter the rheological properties of the olive paste, facilitating the mechanical extraction process and improving phase separation, although some quality parameters may be modified (Rakesh and Sharma, 2007; Mortabit *et al.,* 2014; Moustakime *et al.,* 2016). In addition to using enzymes to increase oil extraction from olive fruits, they have been used to increase oil extraction from other sources such as almond cake as well as fruit juices (Thaiza *et al.,* 2020).

The most important criterion for high-quality olive oil is the content of natural antioxidants, such as tocopherol and phenolic compounds, which can extend the oil shelf-life by slowing the induction step of the oxidation process, in addition to human health benefits (Vitagloine *et al.,* 2015). Furthermore, phenolic compounds have positive effects on the sensory properties of the oil (Nicola, 2016; Zullo *et al.,* 2020).

In Jordan, olive is considered one of the most important agricultural crops, with an annual production of about 250.000 tons of olive fruits and 35.000 tons of olive oils from 20 million trees cultivated in 130.000 hectares (Ministry of Agriculture of Jordan, 2017). About 10 years ago, the Jordanian olive oil yield met the quantities (about 30.000 tons) sufficient to cover the national requirements, enabling growers to export some of those quantities. Many varieties of olive oil are grown in Jordan, but the predominant indigenous varieties are “Nabali Baladi (NB)”, with an oil content of about 20-35%, and “Nabali Muhassan (NM)”, with an oil content of 20-25% (AL-Maaitah *et al.,* 2009; Al-Ismail *et al.,* 2011).

This study aimed to study the influence of two types of exogenous enzymes (added during malaxation) on the extractability of olive oil at early ripening stages from Jordanian cultivars (NB and NM) and to evaluate the effect of enzymes on the quality of virgin olive oil, based

on free acidity, peroxide value, the content of total polyphenols, tocopherol, and pigments, and oxidative stability.

MATERIALS AND METHODS

Materials:

Two Jordanian cultivars of olive, NB, and NM, were subjected to treatment with cellulase and pectinase, and the extracted oil was evaluated in terms of both quality and quantity. The two enzymes were available commercially in powdered forms from Sigma (C1184-5KU, Lot# SLBP8440V), and pectinase (from *Aspergillus niger*, Sigma 17389-50G, Lot# BCBR8237V) was purchased locally.

Sample preparation:

Thirty-eight kilograms of healthy olive fruits from crop season 2017-2018 from each cultivar were collected randomly from approximately 20 trees at the green immature stage, with maturity indexes of about 3.0 and 3.2 for NB and NM, respectively. The olive fruits were picked manually from olive tree orchards located in the north of Jordan (Bani Kenaneh district). Sampling was carried out in triplicate at 2-day intervals (38 kg × 3 for each cultivar at 2-day intervals).

The collected olive fruits were divided into 19 batches (each 2 kg), the first batch (2 kg) was used as a control, while the remaining 18 batches were treated with the selected enzymes. The first 6 batches were treated with cellulase, the second 6 batches with pectinase, and the third 6 batches with a combination (1:1) of both enzymes. The enzymes were added at the following percentages (w/w): 0.02%, 0.04%, 0.06%, 0.08%, 0.10%, and 0.12%, based on a review of similar (Najafian *et al.,* 2009; Rakesh *et al.,* 2015).

Oil was extracted by simulating the traditional method in the laboratory, which starts by crushing the washed olive fruits, then placing the paste in a piece of cloth, then applying pressure using a simple hydraulic press, and then separating the oil from the water using a centrifuge apparatus. In detail: each 2 kg of olive fruits were washed and then crushed using a Hobart mincer (Hobart, London) that provided a 5 mm diameter sieve; the fruit paste was then slowly mixed (15 rpm) for 45 min at 25 °C; the enzyme was added at this point in an aqueous state (dissolved in about 10-20 ml of distilled water) at the beginning of the mixing process (45 minutes).

Oil was obtained by the traditional olive press method as follows. The olive fruit paste was transferred into 16 single-use, disk-shaped cloth mats. The olive paste was spread on the cloth mats and then placed between a stainless-steel piston stacked on top of each other, forming a pile with every 4 mats being separated by a metal disc. The diameters of the cloth mats and metal disks were slightly smaller than that of the piston. Hydraulic pressure was applied on the disks at 120 k N/cm^2 for 45 min; the percolation liquid (oil and vegetation water) was collected and then centrifuged at 5000 rpm for 20 min to separate the oil. A control batch was processed with no enzyme pre-treatments. The previous processing steps were performed in triplicate for each treatment for the NB and NM olive fruits, each batch was 2 kg.

Total oil content and oil extraction:

The olive oil content in NB and NM cultivars was estimated on a dry matter basis, with a Soxhlet fat extraction apparatus using petroleum ether (bp: 40 °C - 60 °C) as the solvent. The extractability for all treatments was calculated by the following formula (AOCS, 1989):

$$\text{Extractability} = \frac{\text{Mass of extracted oil by hydraulic methods}}{\text{Mass of oil content in the control extracted by Soxhlet method}} \times 100$$

Quality standard determinations:

Acidity, peroxide value, and extinction coefficients at wavelengths 232 and 270 nm were determined according to the official methods of the European Union Commission (1991), which are as follows:

- Acidity value was expressed as percent oleic acid and determined by titrating a solution of oil sample in ethanol/ether 1:1 with ethanolic potash.

- Peroxide value was determined following the official methods of the European Union Commission (1991). Peroxide value expressed as $mEqO_2$/kg of oil. Briefly, a mixture of oil samples was dissolved in chloroform/acetic acid 2:3 and left to react in darkness with saturated potassium iodide solution liberated free iodine then titrated with sodium thiosulfate solution in the presence of starch indicator. The peroxide value was calculated as follows:

$$\textbf{Peroxide value} = \frac{\textbf{(A-B)} \times \textbf{N}}{\textbf{W}} \times \textbf{1000}$$

where:

Peroxide value = mEq peroxide per kg of sample

A= volume of titrant (ml) for sample

B = volume of titrant (ml) for blank.

N = normality of $Na_2S_2O_3$ solution (mEq/ml).

1000 = conversion of units (g/kg)

W = Sample mass (g)

-UV extinction coefficient was determined at wavelengths 232 nm and 270 nm. Briefly, K232 and K270 extinction coefficients (absorption of 1% solution in cyclohexane at 232 nm and 270 nm, respectively, with 1 cm of pass length) were measured using a UV spectrophotometer (Spectro UV-VIS Double beam PC, UVD-2950; Labomed, INC. USA).

- Total phenolic content (TP): TP was determined by using the Folin-Ciocalteu colorimetric method (Gutfinger, 1981). Briefly, 10 g olive oil was dissolved in 50 ml hexane, followed by the addition of 20 ml of aqueous methanol (60%) and vigorous mixing for 2 min. The methanolic phase was removed and placed in a beaker each time after the two phases were separated. The combined extracts were laid out to dry in a vacuum rotary evaporator at 70 ºC. The residue was dissolved in 1 ml methanol. A one-tenth milliliter of the methanolic extract was placed into a 10 ml volumetric flask. Five milliliters of distilled water and 0.25 ml Folin-Ciocalteu (2N) were added and mixed well for 3 min. One milliliter of sodium bicarbonate (35% Na_2CO_3) was added and the flask was filled with distilled water up to the mark. The specific absorbance of the blue color formed was measured after 1 h at 725 nm (Spectro UV-VIS Double beam PC, UVD-2950; Labomed, INC. USA). A reference curve was prepared using gallic acid as the most representative of the phenolic standards and the data has been expressed as mg gallic acid/kg of oil (Gutfinger, 1981).

- Tocopherols: These were quantified by High-Performance Liquid Chromatography (HPLC) following a previously reported method (American Oil Chem. Soc. (AOCS, 1989). The Liquid Chromatography (LC) system used was the Knauer system, where a Binary pump Knauer was used, with micro autosampler with a UV detector (Knauer and Smartline 2500 UV Detector (Advanced Scientific Instrument, Berlin, Germany). Olive oil samples were dissolved in 0.36% *n*-hexane (w/v), and 20 µL of the solution was injected into a column (thermoQuest, 10 µ particle size, 4.0 mm ID×30 cm). The mobile phase was hexane-isopropanol (99:1). The flow rate was 1.3 ml/min. The wavelength was programmed at 295 nm.

- Chlorophyll pigment: This was evaluated according to a previously reported method (European Union Commission, 1991). Briefly, the spectrophotometer (Spectro UV-VIS Double beam PC, UVD-2950; Labomed, INC. USA) cell was filled with oil heated to 30 °C and the absorbance was read at 630 nm, 670 nm, and 710 nm, using carbon tetrachloride as blank. The results were calculated by the following equation:

$$\text{Chlorophyll (mg/kg)} = \frac{A_{670} - (A_{630} + A_{710}) / 2}{0.101\ L}$$

where,

A: Absorbance. L: Thickness of cuvette (1 cm)

Carotene pigment: This was measured according to the (AOCS, 1989) method. The absorbance of oil diluted in cyclohexane was measured at wavelength 445 nm using a spectrophotometer (Spectro UV-VIS Double beam PC, UVD-2950; Labomed, INC. USA). The proportion of carotenoids was expressed by β-carotene content calculated using the following equation:

β-carotene (mg/kg) = 383E / PC

where,

E: The difference in measured absorbance values for oil sample and cyclohexane.

P: Optical path length (cm), C: Concentration of the sample (g/100 ml).

- Oxidative stability: Oxidative stability was assessed according to the European Union Commission (1991) method, by the "Rancimat" method (892 Professional Rancimat, Swiss-made), which measured the time (in hours) of resistance to oxidation of 5 g of oil sample exposed to a heat stream of air (22 L/h with 115 °C) passing through the oil sample.

Statistical analysis: All experimental data obtained have been expressed as mean ±SD were carried out in triplicate. The data were analyzed by using the Statistical Package for the Social Sciences (SPSS), version 19.0, 2010, Chicago. IL. Analysis of variance (ANOVA) of the different treatments was done using a randomized complete block design (RCBD) followed by the least significant difference (LSD) test, whose P-value of ≤0.05 was statistically significant.

RESULTS AND DISCUSSION

The olive oil contents of the NB and NM varieties of olive fruit were 48.84% and 42.11%, respectively, expressed as percent on a dry matter basis. The oil extractability of the enzymatically treated and untreated control varieties was 74.60% and 69.15%, respectively. These results were in agreement with the findings of Al-Rousan (2017) and AL-Maaitah et al. (2009) for the same olive cultivars.

As shown in Table 1, there was a significant increase ($P < 0.05$) in the percent extractability of oil extracted from enzyme-treated fruits compared with that of the untreated control samples.

The maximum increases for NB were 4.38%, 3.29%, and 5.25% for those samples treated with cellulase, pectinase, and 1:1 combination of both enzymes, respectively. The increases for NM were 3.86%, 3.09%, and 4.27%, respectively, for the samples treated with cellulase, pectinase, or a 1:1 combination of both enzymes. However, the increases in extractability percentage for both varieties are probably because of effective enzymatic activity on the cell wall of the fruit and the ability to facilitate the breakdown of the emulsion system. These results are in agreement with those obtained by other researchers, where various commercial enzymes such as Ultra SP-L, Pectinase 1.6021, polygalacturonase, β- glucanase, and proteolytic enzymes were used during the malaxation process (Rakesh and Sharma, 2007; Najafian, *et al.,* 2009; Iconomou *et al.,* 2010; Yusoff *et al.,* 2015; Chih *et al.,* 2012). The extractability yield after using a 1:1 combination of the 2 enzymes was significantly ($P < 0.05$) higher at all concentrations compared with that of the control for both olive fruit cultivars. Our results corroborate those of previous studies (Najafian et al., 2009; Rakesh et al., 2015). Such an increase in yield may be attributed to the effect of the combination of the two enzymes in breaking up the colloidal system and emulsion structures of olive fruit paste (Najafian *et al.,* 2009; Chih *et al*., 2012). There was a significant ($P < 0.05$) increase in oil extractability (for both olive cultivars) with the increase in concentrations of cellulase and pectinase. For NB, the

maximum efficiency was observed at a concentration of 0.08%, 0.12%, and 0.12% of cellulase, pectinase, and 1:1 enzyme combination, respectively. However, the increase in extractability in NM was observed at concentrations of 0.1%, 0.12%, and 0.12% using cellulase, pectinase, and a 1:1 combination of both enzymes, respectively.

Table 1 : Percent extractability of oil after treatment with two enzymes and a combination of both at different concentrations by using the pressing extraction method. †

Nabali Baladi						
Enzyme treatment	**0.02**	**0.04**	**0.06**	**0.08**	**0.10**	**0.12**
Cellulase	$_{b}74.8^{c}\pm0.9$	$_{b}75.0^{bc}\pm0.9$	$_{a}76.6^{b}\pm1.0$	$_{a}79.0^{a}\pm0.9$	$_{b}78.6^{a}\pm1.0$	$_{b}78.7^{a}\pm1.0$
Pectinase	$_{b}74.4^{bc}\pm0.9$	$_{b}74.7^{bc}\pm0.9$	$_{a}75.8^{b}\pm1.0$	$_{b}75.7^{b}\pm1.0$	$_{c}77.2^{a}\pm1.0$	$_{bc}77.8^{a}\pm1.0$
Cellulase + Pectinase	$_{a}75.5^{bc}\pm1.0$	$_{a}75.7^{b}\pm1.0$	$_{a}79.0^{a}\pm1.0$	$_{a}79.7^{a}\pm0.9$	$_{a}79.0^{a}\pm1.0$	$_{a}79.9^{a}\pm1.0$
Nabali Muhassan						
Cellulase	$_{ab}69.2^{c}\pm0.8$	$_{ab}69.2^{c}\pm0.8$	$_{b}71.0^{b}\pm0.9$	$_{a}729^{ab}\pm0.9$	$_{a}73.2^{a}\pm0.9$	$_{b}73.0^{a}\pm0.9$
Pectinase	$_{ab}69.5^{bc}\pm0.8$	$_{ab}70.1^{b}\pm0.8$	$_{b}71.0^{b}\pm0.9$	$71.6^{ab}\pm0.9$	$_{a}72.2^{a}\pm0.9$	$_{b}72.2^{a}\pm0.9$
Cellulase + Pectinase	$_{a}70.0^{bc}\pm0.9$	$_{a}71.0^{bc}\pm0.8$	$_{a}73.0^{b}\pm0.9$	$73.7^{a}\pm0.9$	$_{a}73.0^{a}\pm0.9$	$_{a}73.4^{a}\pm0.9$

†The control (NB, NM) content of oil was 48.8, 42.1 respectively.
-Each value is the mean of three replicates.
-Values within the same row with the same superscript letters denote no significant ($p > 0.05$) difference between different enzyme concentrations.
-Values within the same column with the same subscript letters denote no significant ($p > 0.05$) difference between different enzyme treatments.

Acidity, peroxide value, and UV extinction coefficients, K_{232} and K_{270}, were determined to assess the effect of enzyme treatments in oils of both NB and NM. As shown in Table 2, there were no significant differences in acidity, peroxide value, and UV extinction coefficients K_{232} and K_{270} among all the treatments and UV-Extinction coefficient K_{232} and K_{270} of all the treatment groups when compared with that of the control, for both varieties of olive cultivars. It is to be noted that all the results met the international olive oil council standard limits (IOOC., 2003). Our findings are consistent with those obtained in many previous studies (Najafian *et al.,* 2009; García *et al*., 2001; Rakesh and Sharma, 2007). However, some researchers have reported that the acidity and peroxide values decreased when using enzymes (Rakesh and Sharma, 2007; Mortabit *et al*., 2014). These differences could be attributed to many reasons such as the experimental design, harvesting period, and conditions of oil extraction.

One of the most distinguished criteria for the quality of olive oil is its content of antioxidant agents, which include the phenolic compounds, tocopherols, chlorophylls, and carotenes (Vitagloine *et al.,* 2015). The olive oil content of previous compounds was dependent on several factors, including cultivars, degree of ripeness, and extraction method (Iconomou *et al.,* 2010; Koprivnjak *et al.,* 2012; Alu'datt *et al.,* 2017; Peres *et al.,* 2017).

Table 3 presents the effects of enzyme treatment on total phenolic compounds, α-tocopherols, chlorophylls, and carotenes of oil extracted from NB and NM cultivars. TP contents in oil extracted from untreated fruits (controls) of NB and NM were 305 mg GAE/kg and 266 mg GAE/kg, respectively

Table 2. Acidity, Peroxide, and UV- Extinction coefficient K232 and K270 for control and samples treated with cellulase, pectinase, and 1:1 of enzyme combination at different concentrations

Control and treatment samples	Acidity (mg KOH/g oil)		Peroxide (meq/kg oil)		Specific extinction at 232nm $E^{1\%}_{1\,cm}$		Specific extinction at 270nm $E^{1\%}_{1\,cm}$	
	NB		NB	NM	NB	NM	NB	NM
Cellulase		**NM**						
Control	0.26±0.01	0.27±0.05	8.2±1.0	7.9±1.2	1.69±0.23	1.71±0.23	0.30±0.09	0.31±0.08
0.02	0.27±0.01	0.27±0.04	8.1±1.1	8.0±1.3	1.70±0.24	1.68±0.22	0.31±0.09	0.31±0.08
0.04	0.25±0.01	0.28±0.06	8.2±1.0	8.2±1.1	1.68±0.21	1.68±0.21	0.29±0.08	0.31±0.07
0.06	0.26±0.02	0.26±0.06	8.3±1.2	8.3±1.4	1.70±0.23	1.71±0.24	0.29±0.08	0.29±0.09
0.08	0.26±0.02	0.27±0.05	8.2±1.0	8.2±1.4	1.70±0.21	1.70±0.23	0.28±0.09	0.28±0.09
0.10	0.25±0.01	0.26±0.06	8.0±1.1	7.9±1.1	1.68±0.22	1.69±0.23	0.29±0.07	0.31±0.08
0.12	0.25±0.02	0.27±0.06	8.1±1.1	8.2±1.3	1.69±0.24	1.69±0.22	0.29±0.08	0.30±0.07
Pectinase								
0.02	0.27±0.01	0.28±0.06	8.0±1.0	8.1±1.4	1.68±0.22	1.68±0.22	0.29±0.09	0.29±0.07
0.04	0.28±0.02	0.26±0.05	8.2±1.1	8.2±1.3	1.68±0.23	1.68±0.21	0.28±0.07	0.29±0.07
0.06	0.27±0.02	0.26±0.04	8.1±1.2	8.0±1.2	1.69±0.24	1.71±0.23	0.30±0.09	0.29±0.08
0.08	0.26±0.01	0.25±0.05	8.0±1.1	8.1±1.4	1.71±0.24	1.70±0.23	0.30±0.08	0.30±0.08
0.10	0.25±0.01	0.26±0.05	8.2±1.1	8.0±1.3	1.68±0.21	1.69±0.21	0.28±0.08	0.29±0.07
0.12	0.27±0.01	0.27±0.06	8.1±1.1	8.1±1.3	1.69±0.23	1.69±0.21	0.27±0.09	0.31±0.07
Cell + Pectinase 1:1								
0.02	0.28±0.02	0.26±0.06	8.2±1.2	8.2±1.4	1.68±0.22	1.71±0.22	0.30±0.09	0.29±0.08
0.04	0.27±0.02	0.27±0.03	8.1±1.1	8.1±1.3	1.67±0.21	1.67±0.24	0.31±0.09	0.29±0.08
0.06	0.24±0.01	0.27±0.05	8.0±1.2	8.2±1.4	1.69±0.21	1.69±0.23	0.32±0.10	0.27±0.07
0.08	0.25±0.01	0.27±0.04	8.1±1.1	8.0±1.4	1.70±0.23	1.70±0.22	0.30±0.09	0.29±0.09
0.10	0.26±0.02	0.25±0.05	8.0±1.2	8.1±1.2	1.67±0.22	1.69±0.23	0.31±0.08	0.30±0.09
0.12	0.27±0.02	0.26±0.05	8.0±1.1	8.0±1.3	1.68±0.21	1.70±0.22	0.32±0.09	0.31±0.09

*** Each value is the mean of three replicates.**

It has been shown by other researchers that the TP values varied widely from 100 GAE/kg to 937 GAE/kg (Gómez-Rico *et al.*, 2006; Moustakime *et al.,* 2016). A wider range of TP content may be attributed to many reasons including the harvesting period and extraction methods. TP contents in oils extracted from enzyme-treated olive fruits were significantly ($P < 0.05$) higher than that of the control. As shown in Table 3, TP contents of NB were 312-372 mg GAE/kg, 322-400 mg GAE/kg, and 330-425 mg GAE/kg for samples treated with cellulase, pectinase, and 1:1 enzyme combination, respectively. The TP contents of NM were 271-320

GAE/kg, 278-331 GAE/kg, and 284-366 mg GAE/kg for samples treated with cellulase, pectinase, and 1:1 enzyme content of the extracted oil. These results are consistent with those reported earlier by other researchers who intentionally used enzymes to improve olive oil extraction and quality (Iconomou *et al*., 2010; Moustakime *et al.,* 2016; Peres *et al.,* 2017).

α-Tocopherol, the most abundant type of tocopherol found in olive oil, has a significant influence on the oxidative stability of olive oil. It has been reported that the α-tocopherol content of olive oil ranges between 151 mg/kg and 370 mg/kg (Al-Rousan 2017; Al-Ismail *et al.,* 2017; Peres *et al.,* 2017). It is well known that the content of α-tocopherol decreases as the olive ripening progresses (Deiana *et al.,* 2002; Peres *et al.,* 2017).

As shown in Table 3, the α-tocopherol content in untreated samples (controls) of oil extracted from both NB and NM was 189 mg/kg and 163 mg/kg, respectively. combination, respectively. It can be concluded that the enzyme treatment upon malaxation may increase the TP The content of α-tocopherol in enzyme-treated NB samples at different concentrations was 188-248 mg/kg, 194-261 mg/kg, and 195-270 mg/kg for cellulase, pectinase, and 1:1 enzyme combination, respectively. The content of α-tocopherol in enzyme-treated NM samples at different concentrations was 161-219 mg/kg, 174-242 mg/kg, and 185-246 mg/kg for cellulase, pectinase, and 1:1 enzyme combination, respectively. It is evident that there are significant ($P < 0.05$) increases in the α-tocopherol content in enzyme-treated samples. Our results are in agreement with those of earlier studies, which have affirmed the increase in the content of α-tocopherols in olive oil extracted using enzyme treatment of the olive paste during the malaxation process (Ranalli *et al.,* 2003; Bahar *et al*., 2008).

Table 3 Effect of enzyme treatment on the total phenolic compounds, α-tocopherols, chlorophyll and carotene in oil extracted from NB and NM cultivars.

Contr. and tream. samp.	Phenolic compounds (mg gallic acid equiv/kg oil)		α- Tocopherol mg/Kg		Chlorophyll mg/Kg		Carotene mg/Kg	
	NB	NM	NB	NM	NB	NM	NB	NM
Cellulase								
Contr.	$305^{c}\pm8$	$266^{c}\pm6$	$189^{c}\pm6$	$163^{c}\pm6$	$13.81^{c}\pm0.21$	12.13 ± 0.25	$8.72^{ab}\pm 0.08$	$10.10^{b}\pm0.09$
0.02	$312^{c}\pm8$	$271^{b}\pm6$	$188^{c}\pm5$	$161^{c}\pm4$	$13.76^{c}\pm0.22$	$12.21^{bc}\pm0.31$	$8.52^{ab}\pm 0.08$	$10.23^{ab}\pm0.10$
0.04	$325^{b}\pm8$	$275^{b}\pm6$	$202^{cc}\pm5$	$177^{b}\pm4$	$14.21^{bc}\pm0.30$	$12.70^{b}\pm0.29$	$8.71^{ab}\pm 0.09$	$10.33^{a}\pm0.11$
0.06	$338^{b}\pm8$	$284^{b}\pm6$	$213^{c}\pm5$	$189^{b}\pm4$	$15.00^{bc}\pm0.31$	$13.52^{b}\pm0.32$	$8.95^{ab}\pm 0.09$	$10.41^{a}\pm0.11$
0.08	$357^{a}\pm8$	$302^{a}\pm7$	$230^{b}\pm5$	$208^{a}\pm5$	$15.91^{b}\pm0.32$	$14.73^{ab}\pm0.33$	$9.42^{a}\pm1.00$	$10.74^{a}\pm0.12$
0.10	$363^{a}\pm8$	$312^{a}\pm6$	$243^{a}\pm5$	$214^{a}\pm5$	$16.00^{a}\pm0.30$	$15.31^{a}\pm0.33$	$9.51^{a}\pm 1.00$	$10.85^{a}\pm0.11$
0.12	$372^{a}\pm9$	$320^{a}\pm7$	$248^{a}\pm5$	$219^{a}\pm5$	$16.08^{a}\pm0.31$	$15.57^{a}\pm0.34$	$9.50^{a}\pm1.00$	$10.98^{a}\pm0.13$
Pectinase								
0.02	$322^{c}\pm7$	$278^{bc}\pm 7$	$194^{c}\pm5$	$174^{c}\pm41$	$13.90^{c}\pm0.22$	$12.45^{c}\pm0.25$	8.91 ± 0.09	$10.33^{ab}\pm0.12$

0.04	341^{b}±7	289^{b}±7	211^{b}±5	181^{c}±4	14.21^{c}±0.24	13.46^{c}±0.28	8.99± 0.09	10.60^{ab}±0.12
0.06	364^{b}±7	300^{b}±7	222^{b}±5	201^{b}±4.3	15.17^{b}±0.21	14.65^{b}±0.30	9.22±0.09	10.77^{ab}±0.12
0.08	382^{a}±7	317^{ab}±7	239^{ab}±5	220^{ab}±4	16.21^{a}±0.30	15.59^{a}±0.31	9.53±0.09	11.03^{a}±0.13
0.10	391^{a}±8	324^{a}±7.0	248^{ab}±5	231^{a}±4	16.38^{a}±0.31	15.82^{a}±0.32	9.51± 0.10	11.11^{a}±0.14
0.12	400^{a}±8	331^{a}±7	261^{a}±5	242^{a}±4.1	16.56^{a}±0.30	16.00^{a}±0.34	9.62±0.10	11.25^{a}±0.12
Cellulase + Pectinase 1:1								
0.02	330^{e}±8	284^{e}±7	195^{d}±5	185^{c}±5	14.18^{bc}±0.25	13.08^{c}±0.29	9.11^{c}±0.10	10.41^{ab}±0.14
0.04	351^{c}±8	306^{c}±7	219^{bc}±5	194^{bc}±5	14.87^{b}±0.28	13.99^{c}±0.31	9.25^{bc}±0.11	10.55^{ab}±0.13
0.06	373^{b}±8	329^{bc}±7	233^{b}±5	212^{b}±6	15.08^{b}±0.31	14.71^{b}±0.33	9.38^{bc}±0.10	10.72^{ab}±0.13
0.08	398^{a}±8	348^{b}±7	240^{b}±5	232^{ab}±5	16.11^{a}±0.28	15.82^{a}±0.34	9.84^{a}±0.12	11.13^{a}±0.14
0.10	416^{a}±8	357^{a}±8	263^{a}±5	241^{a}±5	16.37^{a}±0.22	16.00^{a}±0.37	9.89^{a}±0.14	11.34^{a}±0.15
0.12	425^{a}±8	366^{a}±8	270^{a}±5	246^{a}±4.7	16.60^{a}±0.27	16.10^{a}±0.35	9.92^{a}±0.11	11.41^{a}±0.14

***** The results in the table represent the average values of the means of 3 runs ± S.D.

*****Values within the same column with same superscript letters denote no significant ($p > 0.05$) difference between values in different individual enzymes treatment.

The contents of chlorophyll and carotene increased significantly ($P < 0.05$) in the enzyme-treated samples compared with those in control samples, as shown in Table 3. The same results were obtained by (Ranalli et al., 2005). The chlorophyll content in untreated samples of oil extracted from NB and NM was 13.81 mg/kg and 12.13 mg/kg, respectively. The carotene content in untreated samples of oil extracted from NB and NM was 8.72 mg/kg and 10.10 mg/kg of oil, respectively. As described previously (Mínguez-Mosquera *et al.,* 1990; Gandul-Rojas *et al.,* 2000), the chlorophyll and carotene contents in olive oil were in the ranges of 1-40 mg/kg and 2-20 mg/kg, respectively.

After treating NB and NM with cellulase (at different concentrations), the chlorophyll content in NB was in the range of 13.76-16.08 mg/kg, whereas in NM, the range was 12.21-15.57 mg/kg. The chlorophyll content of pectinase-treated samples was in the range of 13.90-16.56 mg/kg for NB, and 12.45-16.00 mg/kg for NM. The chlorophyll content in oil extracted from 1:1 enzyme combination-treated samples was 14.18-16.60 mg/kg and 13.08-16.10 mg/kg for NB and NM, respectively. It is noticeable that the increase in chlorophyll content was significant ($P < 0.05$) after the enzyme treatment.

The content of carotenes in enzyme-treated NB reached 9.50 mg/kg, 9.62 mg/kg, and 9.92 mg/kg at the maximum concentration (0.12%) of samples treated with cellulase, pectinase, and 1:1 enzyme combination, respectively. Similarly, the carotene content in the enzyme-treated NM reached 10.98 mg/kg, 11.25 mg/kg, and 11.41 mg/kg at the maximum concentration (0.12%) in those treated with cellulase, pectinase, and 1:1 enzyme combination, respectively (Table 3). The results showing a significant increasing trend in the chlorophyll and carotene contents conform to those obtained by (Ranalli *et al.,* 2005) and (Iconomou *et al.,* 2010), who suggested that the enzyme preparations may facilitate release of pigments from olive fruit tissues. The increases in chlorophyll content demonstrate considerable improvement in oil quality in terms of oxidative stability of the oil (Sergio *et al*., 2007).

The most important quality criterion of olive oil is its antioxidant capacity (Al-Rousan, 2017; Al-Ismail *et al.,* 2017; Peres *et al.,* 2017). This property is mainly influenced by the antioxidant compound content

including that of tocopherol, phenolic compounds, chlorophylls, and carotenes, which provide olive oil with high resistance to oxidation (Gandul-Rojas *et al.,* 2000; Moustakime *et al.,* 2016; Al-Rousan, 2017). The data in Table 3 reveal that the enzymatic treatment may lead to increases in these antioxidant compounds, which confirms their positive effect on the oxidative stability of the oil in both cultivars, as shown in Table 4. The increase in total polyphenols content of the extracted oil could be due to the assistant extraction effect of cellulase and pectinase enzymes that have the capability to break and weaken the cell wall of olive fruits enabling more release of these bound phytochemicals (Aires, 2017).

The oxidative resistance for untreated samples of NB and NM was 50.12 h and 35.77 h, respectively (Table 4). As a consequence of enzymatic treatment by cellulase, these values reached maximum enzyme concentrations (0.12% for cellulase, pectinase, and 1:1 combination) in 76.53 h and 53.23 h for NB and NM respectively, which implies a significant increment ($P < 0.05$) of 52.69% and 48.81% for NB and NM, respectively. The influence of pectinase was less effective than that of cellulase in terms of oxidative stability. However, the oxidative resistance increased to 74.11 h and 52.49 h for NB and NM respectively, with percentage increases of 47.87% and 46.74% for NB and NM, respectively.

The oxidative stability of both olive cultivars (NB and NM) was greatly improved when using the 1:1 combination of two enzymes, and it was reached in 80.05 h and 56.33 h, with percentage increases of 59.77% and 57.48%, respectively. It can be concluded that the NB variety is more resistant to oxidation than NM. In general, the results obtained in this study are in good agreement with those reported earlier (García *et al.,* 2001; Delgado-Adámez *et al*., 2014;), where a high correlation between stability of olive oil to oxidation process and its content of antioxidant agents, particularly phenolic compounds, was reported.

Table 4. Oxidative resistance (h) of olive oil extracted from NB and NM after pretreatment with enzymes (cellulase, pectinase, and combination of both)

Treatment	NB		NM	
Control	50.12±0.82^{d}	% increase in oxidative resistance	35.77±0.46^{d}	% increase in oxidative resistance
Cellulase				
0.02	51.4±0.9^{d}	2.5	36.0±0.7^{d}	0.7
0.04	54.4±0.9	8.6	38.5±0.7^{de}	7.5
0.06	58.1±0.9^{c}	16.0	41.0±0.8^{d}	14.6
0.08	63.5±0.9^{b}	26.7	44.4±0.7^{c}	24.0
0.10	71.1±0.9^{a}	42.0	49.6±0.8^{b}	38.6
0.12	76.5±0.9^{a}	52.7	53.2±0.8^{a}	48.8
Pectinase				
0.02	51.0±0.9^{e}	1.8	36.3±0.7^{c}	1.5
0.04	54.0±0.9^{d}	7.7	39.2±0.7^{bc}	12.3

0.06	**56.1±0.9^{c}**	**11.9**	**42.4±0.7^{b}**	**18.4**
0.08	**61.2±0.9^{b}**	**22.2**	**46.0±0.8^{ab}**	**28.7**
0.10	**71.2±1.0^{a}**	**42.0**	**49.6±0.7^{a}**	**38.6**
0.12	**74.1±1.00^{a}**	**47.9**	**52.5±0.8^{a}**	**46.7**
Cellulase + Pectinase 1:1				
0.02	**52.0±0.9^{d}**	**3.8**	**37.1±0.7^{cd}**	**3.8**
0.04	**57.4±0.9^{d}**	**14.3**	**41.2±0.7^{c}**	**15.2**
0.06	**69.9±0.9^{c}**	**39.4**	**47.8±0.7^{b}**	**33.7**
0.08	**76.1±1.0^{b}**	**51.9**	**53.0±0.8^{ab}**	**48.1**
0.10	**78.3±1.0^{a}**	**56.3**	**55.9±0.7^{a}**	**56.3**
0.12	**80.1±1.0^{a}**	**59.8**	**56.3±0.8^{a}**	**57.5**

The results in the table represent the average values of the means of 3 replicates ± SD. Values within the same column with same superscript letters denote no significant ($p > 0.05$) difference between values in different individual enzyme treatments.

CONCLUSIONS:

We conclude that oil extractability and oil quality from both NB and NM olive fruit cultivars could be improved significantly after enzymatic treatment. Cellulase was more effective than pectinase in increasing oil extractability of both olive cultivars. Using a 1:1 combination of both cellulase and pectinase could be more effective than using individual enzymes. In addition, the qualitative standard parameters in terms of acidity, peroxide content, and UV extinction coefficients K_{232} and K_{270} could be maintained. Additionally, the oxidative stability of oil from both cultivars could be enhanced significantly.

Based on these results, we recommend the use of enzymatic treatment, particularly 1:1 cellulase and pectinase preparation, to improve the quantity and quality of olive oil extracted from NB and NM olive cultivars. It is worthwhile to mention that the application of these findings can help satisfy market demands efficiently.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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استخدام المستحضرات الإنزيمية لتحسين إنتاجية وجودة زيت الزيتون

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ملخص

يعتبر تحسين كمية ونوعية زيت الزيتون المستخرجة أمرا هاما بالنسبة للمنتجين ليس فقط في الأردن ولكن أيضا في جميع أنحاء العالم. هدفت هذه الدراسة إلى تعزيز إنتاجية زيت الزيتون دون المساس بجودته من خلال تطبيق المعالجة الإنزيمية خلال مرحلة التقليب (الخلط) أثناء إستخراج الزيت. والانزيمات التي استخدمت في هذه الدراسة هي السيليوليز والبكتينيز ومزيج من كليهما بنسبة (1:1). تمت إضافة الإنزيمات بتركيزات 0.02٪ و0.04٪ و0.06٪ و0.08٪ و0.10٪ و0.12٪ (وزن/وزن). تم إختيار إثنين من أصناف الزيتون الأردنية، نبالي بلدي (NB) ونبالي محسن (NM)، وكانت ثمار الزيتون لكلا الصنفين و التي خضعت للمعاملة الأنزيمية في مرحلة عدم النضج التام. زاد إنتاج زيت الزيتون بشكل معنوي ($P < 0.05$) بعد المعاملة الإنزيمية. وكانت الزيادات في نسبة الاستخراج 4.38% (على تركيز إنزيم قدره 0.08%)، و 3.29% (على تركيز 0.1%)، و 5.25% (على تركيز 0.12 %) في عينات الزيتون النبالي NB التي عولجت مع السيليوليز ، البكتينيز، ومزيج بنسبة 1:1 من كليهما على التوالي. اما الزيادة المعنوية ($p< 0.05$) في نسبة الاستخلاص للزيت من ثمار الزيتون النبالي المحسن NM فكانت 4.08٪ (على تركيز 0.1٪)، و3.09٪ (على تركيز 0.12٪)، و4.5 (على تركيز 0.08٪) والتي تم معاملتها بإنزيم السيلولاز، والبكتينيز، وخليط 1:1 من كليهما، على التوالي. ولقد كانت نسبة الزيادة المئوية أعلى معنويا($P < 0.05$) لثمار الزيتون NB منها في ثمار الزيتون من نوع النبالي المحسن MN. ولم تتأثر معايير الجودة من حيث الحموضة وقيمة البيروكساید ومعاملات الامتصاص للأشعة فوق البنفسجية عند 232 و270 نانومتر بشكل كبير في أي من مجموعات المعالجة بالمقارنة مع عينات الشاهد. كان محتوى المركبات الفينولية والفا–توكوفيرول والكلوروفيل والكاروتين أعلى معنويا($P < 0.05$) في كلا صنفي الزيت في جميع المعالجات الإنزيمية بالمقارنة مع عينة الشاهد، مما أدى إلى زيادة الثباتية التأكسدية، وبحسب ما أظهرت التحاليل الرانسيماتية.

الكلمات الدالة: استخلاص زيت الزيتون، المعالجة الإنزيمية، جودة زيت الزيتون، مركبات مضادات الأكسدة.