

## Aqueous extraction of virgin olive oil using industrial enzymes

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In the present study, the effects of olive variety (Kroneiki, Iranian Native Oleaginous and Mission), enzyme type (Pectinex Ultra SP-L and Pectinase 1.6021) and concentration (Zero, low and high concentration) on the yield, total polyphenols, turbidity, colour, acidity, peroxide value and iodine value of three enzyme-treated virgin olive oil were investigated. A 3×2×3 Completely Randomized experimental Design (CRD) with replications was carried out. The enzyme concentration had a highly significant effect ( $p < 0.01$ ) on the yield, colour, turbidity and total polyphenol level of oil, but there were not significant effects ( $p < 0.05$ ) on acidity, peroxide value and iodine value. Colour and phenolic compounds content in the oils showed significant differences ( $p < 0.05$ ) between 13.0% to 62.2% and 13.9% to 72.6 %, respectively, as compared with control. Turbidity was reduced significantly ( $p < 0.01$ ) 25.9% to 67.4 %. On the basis of our results, the yield of oil was significantly ( $p < 0.01$ ) increased (from 0.9% to 2.4%) by using processing aid. Pectinex Ultra SP-L was more effective than Pectinase 1.06021. In the case of applying Ultra Pectinex SP-L, the additional income due to extra recovered oil will be 18.8 times as much production overhead.

**Keywords:** Virgin olive oil; Pectolytic enzymes; Aqueous enzymatic extraction

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### ABSTRACT

In the present study, the effects of olive variety (Kroneiki, Iranian Native Oleaginous and Mission), enzyme type (Pectinex Ultra SP-L and Pectinase 1.6021) and concentration (Zero, low and high concentration) on the yield, total polyphenols, turbidity, colour, acidity, peroxide value and iodine value of three enzyme-treated virgin olive oil were investigated. A 3×2×3 Completely Randomized experimental Design (CRD) with replications was carried out. The enzyme concentration had a highly significant effect ( $p<0.01$ ) on the yield, colour, turbidity and total polyphenol level of oil, but there were not significant effects ( $p<0.05$ ) on acidity, peroxide value and iodine value. Colour and phenolic compounds content in the oils showed significant differences ( $p<0.05$ ) between 13.0% to 62.2% and 13.9% to 72.6 %, respectively, as compared with control. Turbidity was reduced significantly ( $p<0.01$ ) 25.9% to 67.4 %. On the basis of our results, the yield of oil was significantly ( $p<0.01$ ) increased (from 0.9% to 2.4%) by using processing aid. Pectinex Ultra SP-L was more effective than Pectinase 1.06021. In the case of applying Ultra Pectinex SP-L, the additional income due to extra recovered oil will be 18.8 times as much production overhead.

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### INTRODUCTION

The high nutrition value of olive oil is mainly due to its high oleic acid content and low levels of free fatty acids, pigments, hydrocarbons and oxygenated compounds. Due to the high ratio of monounsaturated fatty acids to polyunsaturated fatty acids and to high levels of natural antioxidants (phenols and tocopherol), olive oil is very resistant to peroxidation, forming few free radicals (which are highly toxic and detrimental to health). The world production of olive oil is ca. 3 million metric tons per annum, with Spain being the largest producer (5).

The olive fruit contains about 50 % water, 20 % oil, 20 % carbohydrates (pectic, cellulosic and hemocellulosic substances), organic acids, pigments, phenolic compounds and minerals. 96-98 % of the oil is found in the flesh (mesocarp) and skin (pericarp). Only 2-4% oil is found in the pit (endocarp). In order to effectively recovery oil enclosed in the cell, the cell walls must be destroyed. This may be done by enzymes specific to the breakdown of the individual types of polysaccharides in the cell wall structure. The major polysaccharides in the cell wall of olive fruit were found to be the pectic polysaccharides and the hemicellulosic polysaccharides xyloglucan and xylan (17). Enzymatic processes are potentially useful to the edible oil industries due to their high specificity and low operating temperatures. (7,8,10). The cell wall of plants consists mainly of pectic substances, cellulose, hemicellulose and lignin. Many papers have been published on the effects of enzymes on the extraction and characteristics of olive oil (2,4,9,10,16,17). The enzymes present in the olive fruit are in general deactivated during the oil extraction process or crushing step. Thus, exogenous enzymes must be added to the olive paste during the mixing step to replace deactivated enzymes and to enhance the enzyme activity (11).

The objective of the present program was to investigate the feasibility of using enzymes to increase the yield and quality of olive oil from a number of olive varieties. The effects of enzyme type and concentration on virgin olive oil quality as defined by acidity, peroxide value, turbidity, colour and total polyphenols content of extracted oil are reported.

## MATERIALS AND METHODS

### Materials

Handpicked olive from three varieties (Koroneiki, Iranian Native Oleaginous and Mission) produced on Golestan Province, Iran, which were at good sanitary state and normal ripeness were used. Pectinex Ultra SP-L a pectolytic enzyme preparation from *Aspergillus aculeatus*, was obtained from Novo Nordisk BioChem North America, Inc. Pectolytic and hemicellulolytic activities were specified by the manufacturer as not less than 26000  $\text{PG}/\text{ml}$  (pH 3.5), at 35°C ([http:// www.novozymes.com/en.2001](http://www.novozymes.com/en.2001). Pectinex Ultra SP-L.). Pectinase 1.06021 produced from *Aspergillus niger* (also known as polygalacturonidase) was obtained from Merk Company, Darmstadt, Germany.

### Methods

#### Sample preparation

To process the olive samples the following steps were carried out: (1) cleaning and leaves removal; (2) washing; (3) milling by crusher (Rheinische Strabe 36. D. Hann. Germany, Type SK-1) to obtain a fine paste and kept frozen until use; (4) The temperature of the samples was adjusted to enzyme activity temperature in warm water bath, and then the enzymes were added in the beginning of the kneading step by using suitable doses; (5) kneading of the resultant paste under stirring (60 min, 80 rpm); (6) centrifugation of paste at 4500 g, 20 min (BHG ROTO UNI II, Germany); (8) heating to separation of emulsion (a thin but distinctive emulsion layer between the oil and aqueous phases) into an oil and an aqueous phase; (9) mixing the serum with hexane in order to separate the oil. The solvent was evaporated at 50°C. Reference extractions, without employing the enzyme preparations, were also carried out.

#### Chemical analyses

The percentage of olive paste and husk moisture was determined gravimetrically (1; Method Ca 2c-25 ). The oil content of the dried residue was determined as n-hexane extractables using soxhlet extraction (1; Method Ba 6-84 ). Oil colour was determined using spectrophotometric method (Pharmacia LKB.Novaspect II, England), measuring absorbance at 430, 454, 484 and 670 nm and using the following equation:  $C = 1.29A_{430} + 69.7A_{454} + 41.2A_{484} - 56.4A_{670}$  to estimate the Laviband yellow colour value (1; 1L,19 Methods: Aa 6-38, Cc 13c-50, S 2-64 ). The polyphenols were extracted from the oils according to the method of Vazquez Roncero, Janer del Valle and Janer del Valle (1973). Turbidity was determined as follows,  $T = T_1 - T_2$  where :  $T_1$  is oil turbidity in NTU at 130°C ,  $T_2$  is oil turbidity in NTU at 5.5°C (after 1hr keeping in refrigeration) by helping standard curve (6). Free acidity, peroxide and iodine values were also determined by standard AOCS titrimetric methods Ca 5a-40, Ja 8-87 and Cd 1c-85, respectively.

#### Statistical analyses

A 3×2×3 factorial design (3 olive varieties × two enzyme types × 3 enzyme concentrations) was adopted. Two-sided variance analysis (ANOVA) with replications was used to test for the quantitative and qualitative effects of the enzyme on the oil. Means were separated using Duncan's multiple range test. Probabilities greater than  $p = 0.05$  were considered nonsignificant.

## RESULTS AND DISCUSSION

The chemical composition of the olive varieties (Koroneiki, Iranian Native Oleaginous and Mission) is shown in Table 1. Varietal differences are likely responsible for differences in oil and solid content of the varieties investigated.

### Oil extraction yield

The enzyme treatments resulted in higher overall oil yields, the increases ranged from 0.9 to 2.4%, wet basis (Table 3) which is statistically significant ( $p < 0.01$ ) (Table 2). Based on our results, increasing the enzyme concentration increased oil yield. The best results were obtained with the Koroneiki variety, using Pectinex enzyme at the higher concentration. This increased oil yield would be of very significant value in industrial processing. The extraction yield increases obtained in this study compared favourably with results from other experiments reported earlier (10).

### Total polyphenols

Polyphenols are biologically active components that affect flavour and have antioxidant properties that extend the shelf- life of the product (17). Both of the enzyme treatments increased the phenolic content of the oil (Table 3). The effects of enzymes on total polyphenols was significant ( $p < 0.01$ ) ranging from 18 to 76% of the enzyme-free control (Table 2). The greatest effect was observed in the Koroneiki variety, with least effect on the Iranian Oleaginous variety. Increased enzyme addition levels resulted in higher polyphenol concentrations ( $P < 0.01$ ) as shown in (Fig. 1). The effects of Pectinex and Pectinase enzyme preparations were statistically different ( $P < 0.01$ ). Except for the Iranian Oleaginous variety Pectinex treatment resulted in greater increase in polyphenols in the oil, than the Pectinase (Table 2). These results are consistent with the data reported earlier by Italian researchers which mentioned that addition of the pectolytic enzymes to the oily pastes constantly exhibited higher total polyphenols in general, averaging 18.8% (10).

Vierhuis *et al.* (2003) have shown that the addition of a cell-wall- degrading- enzyme preparation during the mechanical extraction of olive oil can increase the release of phenolic compounds into the oil. The addition of commercial enzyme preparations might have also reduced the complexation of the phenolic compounds with the polysaccharides, thus increasing the concentration of free phenols in the pastes and their release into the oil during processing (12,16).

### Turbidity and Colour

Turbidity and colour are not considered by the EEC method for the evaluation of sensory characteristics of olive oil, but both actually influence consumer acceptability of the product. Therefore, these parameters should be considered for inclusion in testing the oil. We found that the turbidity values were frequently higher in the untreated oils (Table 3) probably owing to the reduced colloidal particle content in the enzyme treated oil. This is in agreement with the results published by Ranalli *et al.*, (12,13,14). Increasing the enzyme content decreased the oil turbidity (Fig. 2), and Iranian Native Oleaginous variety, on which applied higher concentration of enzyme had the highest turbidity reduction of up to 64.2 % (Table 3). The enzyme preparation exerted a significant effect ( $p < 0.01$ ) on the colour of the treated oils (Table 2). We found that the colour of treated oils were higher than those of the control oils (Table 3). Our result showed that the Koroneiki variety, treated with Pectinase had the highest oil colour index (Table 3). The oil colour index of the Mission variety exhibited the largest increase at high enzyme concentration, 59.7%. These finding were confirmed by

values of the integral colour index (Naudet's index) which reported increasing of 12.4%, 6.5% and 25.1% for Dritta, Leccino and Coratina varieties, respectively due to applying enzyme pretreatment, Indicating that oils were characterised by a yellow colour, which clearly prevailed over green. (14). The literature suggests that the enzyme formulation promoted the release of yellow and green lipochromes (Carotenes and Chlorophylls) from the vegetable tissue, thus increasing their solubilization and content in the oil (13).

#### Peroxide value, acidity and iodine value

EC regulation no. 2568 (1991) is used commercially to assess olive oil quality. The regulation defines target values for peroxide value, acidity and iodine value only. As expected the oil quality parameters were dependent only on the seed variety and were not affected by the enzyme treatments (Tables 2 & 3).

Table 1- Compositional characteristics of the three processed olive varieties<sup>a</sup>

Olive variety	Oil (%)	Misture (%)	Solid (%)
Koroneiki	24.3± 0.5	52.5± 2.29	23.2± 2.76
Iranian native Oleaginous	16.2 ± 0.34	63.8± 0.51	20± 0.85
Mission	13.1± 0.26	70.2± 0.45	16.7± 0.57

<sup>a</sup> Data are means of at least three replicates ±SD.

Table 2. Analysis of variance (Mean square) of the effect of various treatments investigated on the virgin olive oil qualitative and quantitative characteristics<sup>a</sup>

Variable	Mean Square			
	Colour	Turbidity	Total Polyphenols	Oil Yield
Variety	597.915**	174.03**	51393.0**	241.412**
Enzyme type	0.623*	46.111*	7072.7**	1.965 <sup>ns</sup>
Enzyme Concentration	25.224**	4551.7**	31319.0**	15.701**
Variety* Enzyme type	0.428*	0.601 <sup>ns</sup>	1987.7**	0.056 <sup>ns</sup>
Variety* Enzyme Concentration	3.822**	135.56***	788.3**	0.318 <sup>ns</sup>
Enzyme type * Enzyme Concentration	0.159 <sup>ns</sup>	12.911 <sup>ns</sup>	1800.2**	0.491 <sup>ns</sup>
Variety* Enzyme type * EnzymeConcentration	0.110 <sup>ns</sup>	1.993 <sup>ns</sup>	529.3**	0.015 <sup>ns</sup>

<sup>a</sup>Values with one or two asterisks are significantly different from the corresponding controls (\* p< 0.05; \*\* p< 0.01).

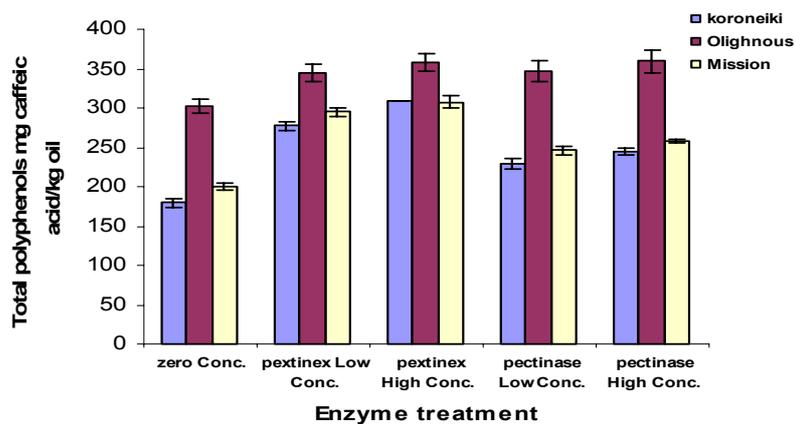
Table 3. Effect of variety, enzyme type and enzyme concentrations on the compositional characteristics of three enzyme-treated virgin olive oils<sup>a</sup>

Sample	Acidity (as oleic acid, %)	Peroxide value (meq o <sub>2</sub> kg <sup>-1</sup> )	Iodine value	Colour index	Turbidity (NTU) <sup>b</sup>	Total Polyphenols (mg caffeic acid/kg oil)	Oil (% fruit oil)
Koroneiki							
Pectinex enzyme							
(Control)	0.25±0.01	1.37±0.05 <sup>cd</sup>	80.4±0.04 <sup>cd</sup>	12.9±0.08 <sup>d</sup>	59.99±2.72 <sup>a</sup>	179±5.35 <sup>b</sup>	69.7±0.45 <sup>b</sup>
Lowconcentration	0.24±0.01	1.3 <sup>cd</sup>	80.49±0.02 <sup>cd</sup>	15.85±0.12 <sup>c</sup>	33.88±2.08 <sup>de</sup>	277.33±4.03 <sup>d</sup>	71.93±0.49 <sup>a</sup>
High concentration	0.24	1.26±0.05 <sup>d</sup>	80.53c±0.02 <sup>c</sup>	16.15±0.04 <sup>bc</sup>	20.55±2.08 <sup>hi</sup>	309±0.82 <sup>c</sup>	72.13±0.49 <sup>a</sup>
Pectinase enzyme							
(Control)	0.25±0.01	1.37±0.05 <sup>cd</sup>	80.4±0.04 <sup>cd</sup>	12.9±0.08 <sup>d</sup>	59.99±2.72 <sup>a</sup>	179±5.35 <sup>b</sup>	69.7±0.45 <sup>b</sup>
Low concentration	0.25	1.4 <sup>c</sup>	80.4±0.04 <sup>cd</sup>	16.7±0.08 <sup>ab</sup>	36.66± 8.82 <sup>d</sup>	229.33±4.92 <sup>f</sup>	71.23±0.49 <sup>a</sup>

High concentration	0.24±0.01	1.37±0.05 <sup>sd</sup>	80.36±0.04 <sup>d</sup>	16.93±0.05 <sup>a</sup>	22.22±5.09 <sup>ghi</sup>	245±4.08 <sup>c</sup>	71.37±0.45 <sup>a</sup>
Iranian oleaginous							
Pectinex enzyme							
(Control)	0.26±0.01	2.17±0.05 <sup>b</sup>	90.77±0.07 <sup>b</sup>	5.63±0.09 <sup>f</sup>	52.77±0.79 <sup>b</sup>	302.33±7.76 <sup>c</sup>	66.07±0.76 <sup>d</sup>
Low concentration	0.25±0.01	2.2±0.08 <sup>b</sup>	90.70±0.06 <sup>b</sup>	6.37±0.05 <sup>e</sup>	27.21±0.77 <sup>fe</sup>	344.33±8.73 <sup>b</sup>	68±0.78 <sup>c</sup>
High concentration	0.27±0.01	2.27±0.05 <sup>b</sup>	90.67±0.07 <sup>b</sup>	6.53±0.05 <sup>e</sup>	17.22±0.79 <sup>i</sup>	357.67±9.18 <sup>ab</sup>	68.1±0.78 <sup>c</sup>
Pectinase enzyme							
(Control)	0.26±0.01	2.17±0.05 <sup>b</sup>	90.77±0.07 <sup>b</sup>	5.63±0.09 <sup>f</sup>	52.77±0.79 <sup>b</sup>	302.33±7.76 <sup>c</sup>	66.07±0.76 <sup>d</sup>
Low concentration	0.27±0.01	2.27±0.05 <sup>b</sup>	90.71±0.08 <sup>b</sup>	6.6±0.14 <sup>e</sup>	29.43±0.8 <sup>ef</sup>	346.67±10.27 <sup>ab</sup>	67.4±0.71 <sup>c</sup>
High concentration	0.27±0.01	2.27±0.05 <sup>b</sup>	90.66±0.08 <sup>b</sup>	6.8±0.14 <sup>e</sup>	20.55±1.57 <sup>hi</sup>	359±12.33 <sup>a</sup>	67.53±0.74 <sup>c</sup>
Mission							
Pectinex enzyme							
(Control)	0.43±0.01	3±0.08 <sup>a</sup>	91.06±0.08 <sup>a</sup>	3.43±0.31 <sup>h</sup>	44.98±1.36 <sup>c</sup>	199.67±3.63 <sup>g</sup>	62.9±0.59 <sup>f</sup>
Low concentration	0.44±0.01	3±0.08 <sup>a</sup>	90.99±0.1 <sup>a</sup>	4.57±0.42 <sup>g</sup>	31.66±1.66 <sup>def</sup>	295.33±4.99 <sup>c</sup>	64.23±0.59 <sup>ef</sup>
High concentration	0.43	3.1±0.08 <sup>a</sup>	90.95±0.09 <sup>a</sup>	5.57±0.77 <sup>f</sup>	21.11±20.8 <sup>hi</sup>	306.67±6.24 <sup>c</sup>	64.33±0.54 <sup>e</sup>
Pectinase enzyme							
(Control)	0.43±0.01	3±0.08 <sup>a</sup>	91.06±0.08 <sup>a</sup>	3.43±0.31 <sup>h</sup>	44.98±1.36 <sup>c</sup>	199.67±3.63 <sup>g</sup>	62.9±0.59 <sup>f</sup>
Low concentration	0.44±0.01	3.1±0.08 <sup>a</sup>	90.99±0.08 <sup>a</sup>	4.57±0.26 <sup>g</sup>	33.32±1.37 <sup>de</sup>	246±4.32 <sup>c</sup>	63.87±0.58 <sup>ef</sup>
High concentration	0.43±0.01	3.07±0.05 <sup>a</sup>	90.94±0.07 <sup>a</sup>	5.9±0.5 <sup>f</sup>	26.09±3.13 <sup>ghi</sup>	258.33±2.36 <sup>c</sup>	64±0.62 <sup>ef</sup>

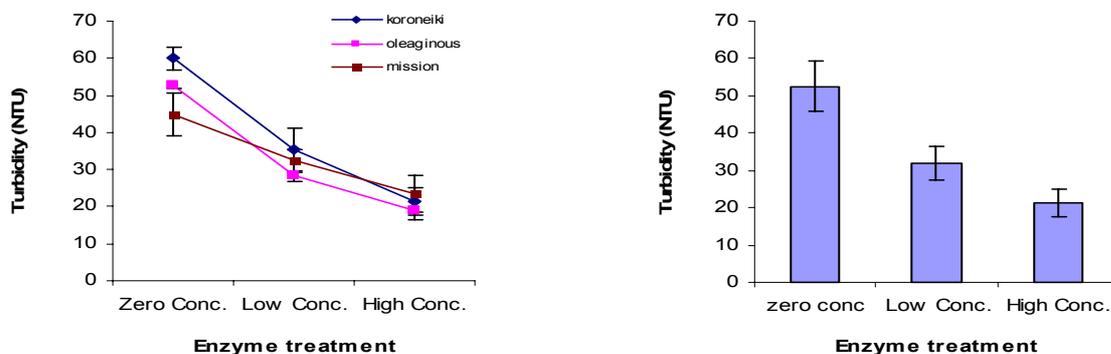
<sup>a</sup>- Results are mean values of at least 3 replicates ± standard deviation. <sup>b</sup>- NTU, nephelometric Turbidity units.

†Column values followed by the same letter are not significantly different ( $P < 0.05$ ).



<sup>a</sup>Error bars indicate standard deviation.

Fig. 1. The effect of variety, enzyme type and concentration on total polyphenol of the three enzyme-treated virgin olive oil <sup>a</sup>.



<sup>a</sup>Error bars indicate standard deviation.

Fig. 3. The effect of enzyme concentration × olive variety (a) and enzyme concentration (b) on the turbidity of the three enzyme-treated virgin olive oil <sup>a</sup>

### CONCLUSIONS

These results demonstrated that oil extraction from olive can be enhanced by enzyme hydrolysis. The enzyme treatment, besides giving higher oil yields, significantly increased the qualitative standard of the three virgin olive oil varieties produced. Pectinex Ultra SP-L was found to be more effective than Pectinase 1.06021. In order to achieve higher yields, it is better to use 0.02% (v/w) of enzyme, but higher enzyme concentrations (0.04% v/w) is needed for a better quality of the extracted oil. Therefore, in the case of applying Ultra Pectinex SP-L the average oil recovery will be increase 1.96%. If so, the production overhead is 1.25\$ per 100 kg olive fruit Whereas, the income will be increase to 23.5\$. In other words, the additional income due to extra recovered oil will be 18.8 times as much production overhead. This will allow the yields and the qualitative standard of the product to be significantly improved.

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