Food Research International 42 (2009) 171–175

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Aqueous extraction of virgin olive oil using industrial enzymes

L. Najafian [a], A. Ghodsvali [b,*], M.H. Haddad Khodaparast [c], L.L. Diosady [d]

[a] Department of Food Science and Technology, Azad University, Sabzevar Branch, Sabzevar, Iran
[b] Department of Agricultural Engineering, Golestan Agricultural and Resources Research Center, Gorgan-Shahid beheshti Ave. P.O.B. 49156-77555, Gorgan, Iran
[c] Department of Food Science and Technology, University of Ferdosi, Mashad, Iran
[d] Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, Ontario, Canada M5S 3E5

ARTICLE INFO

Article history:
Received 12 July 2008
Accepted 1 October 2008

Keywords:
Virgin olive oil
Pectolytic enzymes
Aqueous enzymatic extraction

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 $3 \times 2 \times 3$ 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 13.0–62.2% and 13.9–72.6%, respectively, as compared with control. Turbidity was reduced significantly ($p < 0.01$) 25.9–67.4%. 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.

1. 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 (http://www.fas.USDA.gov/psdonline/psdReport.aspx, 2008).

The olive fruit contains about 50% water, 20% oil, 20% carbohydrates (pectic, cellulosic and hemicellulosic 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). The common methods of olive oil extraction include physical or mechanical processes, chemical procedures or a combination of these. During the conventional oil extraction processes, some of the oil not extracted remains in the solid residue. Several methods have been proposed improving oil extraction procedures including enzymatic pretreatment. The majority of the oil is located in the vacuoles as free oil but oil dispersed in the cytoplasm is not accessible in the extraction process and is therefore lost in the waste (Obergfoll, 1997). In order to effectively recover 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. Vierhuis, Korver, Schols, and Voragen (2003) indicated that the major polysaccharides in the cell wall of olive fruit were found to be the pectic polysaccharides and the hemicellulosic polysaccharides xyloglucan and xylan.

Enzymatic processes are potentially useful to the edible oil industries due to their high specificity and low operating temperatures. Enzyme applications in edible oil processing include: facilitating pressing, increasing the oil yield of solvent extraction, and facilitating the aqueous extraction (Ranalli & De Mattia, 1997; Ranalli & Ferrante, 1996; Ranalli & Lazzari, 1996). The enzymes are able to breakdown the cell structure of plants and to release the oil from cells. 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 (e.g. Domínguez, Núñez, & Lema, 1994; Garcia et al., 2001; Ranalli & De Mattia, 1997; Ranalli & Serraiocco, 1996; Ranalli, Sgaramella, & Surricchio, 1999; Vierhuis et al., 2001; Vierhuis et al.,2003). 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 (Ranalli, De Mattia, & Ferrante, 1998).

* Corresponding author. Tel.: +98 171 33500635x261; fax: +98 171 3359813.
E-mail address: qodsevali@yahoo.com (A. Ghodsvali).

0963-9969/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodres.2008.10.002

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.

2. Materials and methods

2.1. 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 26,000 $^{PG}/_{ml}$ (pH 3.5), at 35 °C (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.

2.2. Methods

2.2.1. 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; and (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.

2.2.2. Chemical analyses

The percentage of olive paste and husk moisture was determined gravimetrically (AOCS, 1993; Method Ca 2_C–25). The oil content of the dried residue was determined as *n*-hexane extractables using soxhlet extraction (AOCS, 1993; Method Ba 6-84). The solid content was calculated as oil and moisture free solids by the formula: 100 − (oil% + moisture%). Oil colour was determined using spectrophotometric method (Phamacia 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 Lovibond yellow colour value (AOCS, 1993; 1L,19 Methods: Aa 6-38, Cc 13_c-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). Ten grams of oil was dissolved in 50 ml *n*-hexane and the solution was extracted successively with three 20 ml portions of 60% aqueous methanol. The mixture was shaken each time for 2 min. The solvent was removed from the combined using a vacuum rotary evaporator (LABOROTA 4001- Efficient, Heidolph Co.) at 40 °C. The residue was dissolved in 1 ml methanol and was stored frozen until the moment of the analyses. The concentration of total polyphenols in the methanolic extract was estimated with Folin Ciocalteau reagent. The procedure consisted of dilution of 0.1–0.4 ml methanolic extract with water to 5 ml in a 10 ml volumetric flask, and addition of 0.5 ml Folin Ciocalteau reagent. After 3 min, 1 ml of saturated (ca. 35%) Na_2CO_3 Solution was added. The content was mixed and diluted to volume (10 ml) with water. The absorbance was measured after 1 h at 725 nm against a reagent blank. Caffeic acid served as a standard for preparing the calibration curve ranging 0–100 μg/10 ml assay solution (Gutfinger, 1981).

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 1 h keeping in refrigeration) by helping standard curve (Ranalli & Constantini, 1994).

Free acidity, peroxide and iodine values were also determined by AOCS (1993) standard methods Ca 5a-40, Ja 8-87 and Cd 1_c-85, respectively.

2.2.3. Statistical analyses

A 3 × 2 × 3 factorial design (3 olive varieties × 2 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.

3. Results and disscusion

The chemical composition of the olive varieties (Koroneiki, Iranian Native Oleaginous and Mission) is shown in Table 1. Varietal

Table 1
Compositional characteristics of the three processed olive varieties[a].

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

[a] 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[a].

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^{ns}
Enzyme concentration	25.224^{**}	4551.7^{**}	31319.0^{**}	15.701^{**}
Variety * enzyme type	0.428^{*}	0.601^{ns}	1987.7^{**}	0.056^{ns}
Variety * enzyme concentration	3.822^{**}	135.56^{***}	788.3^{**}	0.318^{ns}
Enzyme type * enzyme concentration	0.159^{ns}	12.911^{ns}	1800.2^{**}	0.491^{ns}
Variety * enzyme type * enzymeconcentration	0.110^{ns}	1.993^{ns}	529.3^{**}	0.015^{ns}

[a] Values with one or two asterisks are significantly different from the corresponding controls ($^{*}p < 0.05$; $^{**}p < 0.01$).

differences are likely responsible for differences in oil and solid content of the varieties investigated.

3.1. 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). In our experiments, increasing the enzyme concentration tended to increase the oil yield (Table 2, Fig. 1). The best results were obtained with the Koroneiki variety, using pectinex enzyme at the higher concentration (Fig. 1). 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 (Ranalli & De Mattia, 1997).

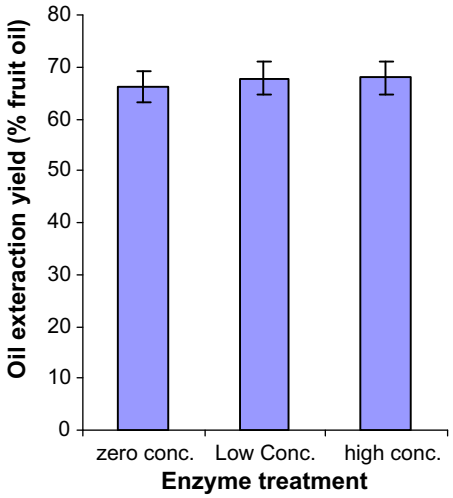

Fig. 1. The effect of enzyme concentration on extraction yield of the three enzyme-treated virgin olive oil. Error bars indicate standard deviation.

Table 3
Effect of variety, enzyme type and enzyme concentrations on the compositional characteristics of three enzyme-treated virgin olive oils[A].

Sample	Iodine value	Total polyphenols (mg caffeic acid/ kg oil)	Oil (% fruit oil)
Koroneiki			
Pectinex enzyme			
(Control)	80.4 ± 0.04^{cd}	179 ± 5.35h	69.7 ± 0.45^{b}
Low concentration	80.49 ± 0.02^{cd}	277.33 ± 4.03^{d}	71.93 ± 0.49^{a}
High concentration	80.53^{c} ± 0.02^{c}	309 ± 0.82^{c}	72.13 ± 0.49^{a}
Pectinase enzyme			
(Control)	80.4 ± 0.04^{cd}	179 ± 5.35^{h}	69.7 ± 0.45^{b}
Low concentration	80.4 ± 0.04^{cd}	229.33 ± 4.92^{f}	71.23 ± 0.49^{a}
High concentration	80.36 ± 0.04^{d}	245 ± 4.08^{e}	71.37 ± 0.45^{a}
Iranian oleaginous			
Pectinex enzyme			
(Control)	90.77 ± 0.07^{b}	302.33 ± 7.76^{c}	66.07 ± 0.76^{d}
Low concentration	90.70 ± 0.06^{b}	344.33 ± 8.73^{b}	68 ± 0.78^{c}
High concentration	90.67 ± 0.07^{b}	357.67 ± 9.18^{ab}	68.1 ± 0.78^{c}
Pectinase enzyme			
(Control)	90.77 ± 0.07^{b}	302.33 ± 7.76^{c}	66.07 ± 0.76^{d}
Low concentration	90.71 ± 0.08^{b}	346.67 ± 10.27^{ab}	67.4 ± 0.71^{c}
High concentration	90.66 ± 0.08^{b}	359 ± 1 2.33^{a}	67.53 ± 0.74^{c}
Mission			
Pectinex enzyme			
(Control)	91.06 ± 0.08^{a}	199.67 ± 3.63^{g}	62.9 ± 0.59^{f}
Low concentration	90.99 ± 0.1^{a}	295.33 ± 4.99^{c}	64.23 ± 0.59^{ef}
High concentration	90.95 ± 0.09^{a}	306.67 ± 6.24^{c}	64.33 ± 0.54^{e}
Pectinase enzyme			
(Control)	91.06 ± 0.08^{a}	199.67 ± 3.63^{g}	62.9 ± 0.59^{f}
Low concentration	90.99 ± 0.08^{a}	246 ± 4.32^{e}	63.87 ± 0.58^{ef}
High concentration	90.94 ± 0.07^{a}	258.33 ± 2.36^{e}	64 ± 0.62^{ef}

Column values followed by the same letter are not significantly different ($p < 0.05$).
[A] Data are means of at least 3 replicates ± standard deviation.

3.2. Total polyphenols

Polyphenols are biologically active components that affect flavour and have antioxidant properties that extend the shelf-life of the product (Servili et al., 1999). 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. 2. 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% (Ranalli & De Mattia, 1997).

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 (Ranalli et al., 1999; Vierhuis et al., 2001).

3.3. 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 4) probably owing to the reduced colloidal particle content in the enzyme treated oil. This is in agree-

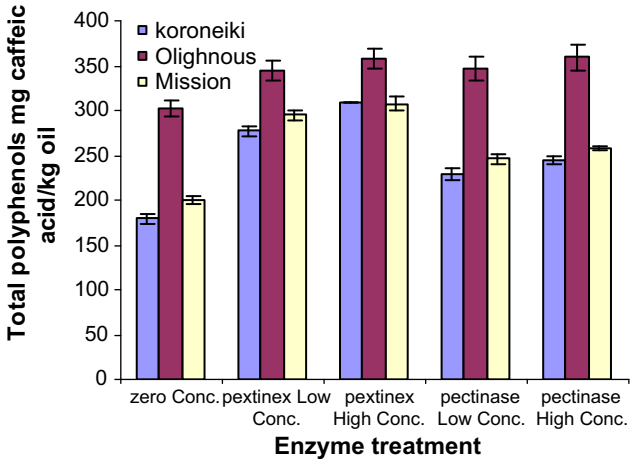

Fig. 2. The effect of variety, enzyme type and concentration on total polyphenol of the three enzyme-treated virgin olive oil. Error bars indicate standard deviation.

Table 4
Effect of variety, enzyme type and enzyme concentrations on the qualitative characteristics of three enzyme-treated virgin olive oils[A].

Sample	Acidity (as oleic acid%)	Peroxide value (meq o_2 kg^{-1})	Colour (Laviband)	Turbidity (NTU)[B]
Koroneiki				
Pectinex enzyme				
(Control)	0.25 ± 0.01	1.37 ± 0.05^{cd}	12.9 ± 0.08^{d}	59.99 ± 2.72^{a}
Low concentration	0.24 ± 0.01	1.3^{cd}	15.85 ± 0.12^{c}	33.88 ± 2.08^{de}
High concentration	0.24	1.26 ± 0.05^{d}	16.15 ± 0.04 ^{bc}	20.55 ± 2.08^{hi}
Pectinase enzyme				
(Control)	0.25 ± 0.01	1.37 ± 0.05^{cd}	12.9 ± 0.08^{d}	59.99 ± 2.72^{a}
Low concentration	0.25	1.4^{c}	16.7 ± 0.08^{ab}	36.66 ± 8.82^{d}
High concentration	0.24 ± 0.01	1.37 ± 0.05^{cd}	16.93 ± 0.05^{a}	22.22 ± 5.09^{ghi}
Iranian oleaginous				
Pectinex enzyme				
(Control)	0.26 ± 0.01	2.17 ± 0.05^{b}	5.63 ± 0.09^{f}	52.77 ± 0.79^{b}
Low concentration	0.25 ± 0.01	2.2 ± 0.08^{b}	6.37 ± 0.05^{e}	27.21 ± 0.77^{fg}
High concentration	0.27 ± 0.01	2.27 ± 0.05^{b}	6.53 ± 0.05^{e}	17.22 ± 0.79^{i}
Pectinase enzyme				
(Control)	0.26 ± 0.01	2.17 ± 0.05^{b}	5.63 ± 0.09^{f}	52.77 ± 0.79^{b}
Low concentration	0.27 ± 0.01	2.27 ± 0.05^{b}	6.6 ± 0.14^{e}	29.43 ± 0.8^{ef}
High concentration	0.27 ± 0.01	2.27 ± 0.05^{b}	6.8 ± 0.14^{e}	20.55 ± 1.57^{hi}
Mission				
Pectinex enzyme				
(Control)	0.43 ± 0.01	3 ± 0.08^{a}	3.43 ± 0.31^{h}	44.98 ± 1.36^{c}
Low concentration	0.44 ± 0.01	3 ± 0.08^{a}	4.57 ± 0.42^{g}	31.66 ± 1.66^{def}
High concentration	0.43	3.1 ± 0.08^{a}	5.57 ± 0.77^{f}	21.11 ± 20.8^{hi}
Pectinase enzyme				
(Control)	0.43 ± 0.01	3 ± 0.08^{a}	3.43 ± 0.31^{h}	44.98 ± 1.36^{c}
Low concentration	0.44 ± 0.01	3.1 ± 0.08^{a}	4.57 ± 0.26^{g}	33.32 ± 1.37^{de}
High concentration	0.43 ± 0.01	3.07 ± 0.05^{a}	5.9 ± 0.5^{f}	26.09 ± 3.13^{fgh}

Column values followed by the same letter are not significantly different ($p < 0.05$).
[A] Data are means of at least 3 replicates ± standard deviation.
[B] NTU, nephelometric turbidity units.

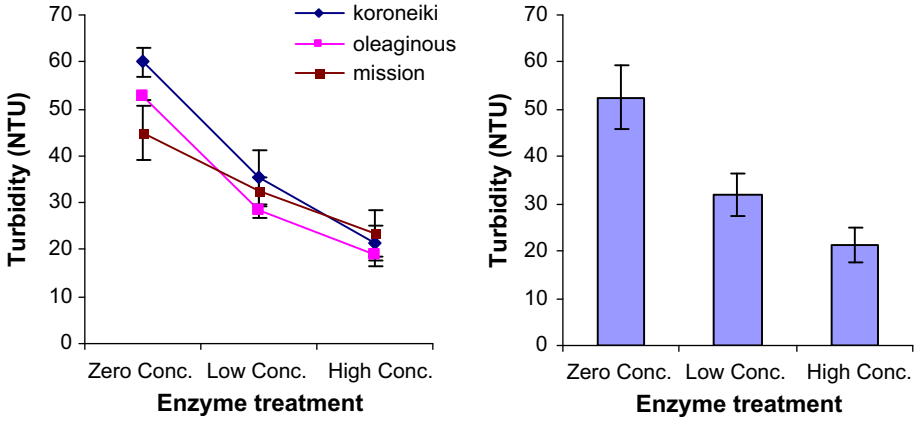

Fig. 3. The effect of enzyme concentration × olive variety (a) and enzyme concentration (b) on the turbidity of the three enzyme-treated virgin olive oil. Error bars indicate standard deviation.

ment with the results published by Ranalli et al. (1999, 2001, 2003). Increasing the enzyme concentration decreased the oil turbidity (Fig. 3). The Iranian native oleaginous variety treated with the higher concentration of enzyme had the highest turbidity reduction 64.2% (Table 4). 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 4). Our result showed that the Koroneiki variety, treated with pectinase had the highest oil colour index (Table 4). The oil colour index of the mission variety exhibited the largest increase at high enzyme concentration, 59.7% (Fig 4). These finding were consistent with increased values of the integral colour index (Naudet's index) reported by Ranalli, Gomes, Delcurator, Contento, and Lucera (2003). Dritta, Leccino and Coratina varieties had their colour values increased by of 12.4%, 6.5% and 25.1%, respectively after enzyme pretreatment. These values indicate that in these oils a yellow colour clearly prevailed over green. 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 (Ranalli, Malfatti, & Cabras, 2001).

3.4. 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

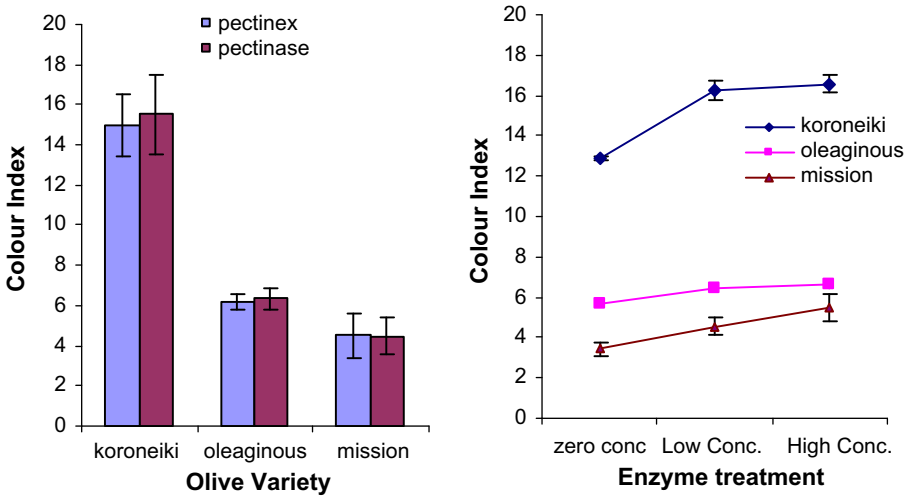

Fig. 4. The effect of variety × enzyme type (a) and variety × enzyme concentration (b) on the colour index of the three enzyme-treated virgin olive oil. Error bars indicate standard deviation.

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 and 4).

4. Conclusions

These results demonstrated that oil extraction from olive can be enhanced by enzyme hydrolysis. It has been demonstrated that pre extraction enzyme digestion increases cellular degradation and significantly increases oil recovery upon extraction. 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. The qualitative and quantitative results achieved lead us to propose that the use of these enzyme formulations be officially recognized in olive-producing countries throughout the world. This will allow the yields and the qualitative standard of the product to be significantly improved.

Acknowledgements

The authors gratefully acknowledge the Iranian Agricultural Engineering Research Institute for its financial support. We also thank Mrs. Roshan for her technical assistance.

References

AOCS (1993). *Official methods and recommended practices of the American oil chemist's society* (5th ed.). Champaign: The American Oil Chemist's Society.

EC, European Community (1991). International trade standard applying to olive oil and olive-residue oils. EC Regulation No. 2568. European Community's Official Gazette No. L 248, p. 10–31.

Domínguez, H., Núñez, M. J., & Lema, Y. M. (1994). Enzymatic treatment to enhance oil extraction from fruit and oilseeds: A review. *Food Chemistry, 49*, 271–286.

Garcia, A., Brenes, M., Moyano, M. J., Alba, J., Garcia, P., & Garcia, A. (2001). Improvement of phenolic compound content in virgin olive oils by using enzymes during malaxation. *Journal of Food Engineering, 48*, 189–194.

Gutfinger, T. (1981). Polyphenols in olive oils. *Journal of the American Oil Chemist's Society, 58*, 966–968.

Obergfoll, H.-M. (1997). *The use of enzymes in extraction of olive oil*. Switzerland: Novo Nordisk Ferment Ltd.

Ranalli, A., Malfatti, A., & Cabras, P. (2001). Composition and quality of pressed virgin olive oils extracted with a new enzyme processing aid. *Journal of Food Science, 66*, 592–603.

Ranalli, A., & Constantini, N. (1994). Olive oil extraction: role of the biotechnologies Note 2. *Riv. Ital. Sost. Grasse., 71*, 399–407.

Ranalli, A., & De Mattia, G. (1997). Characterisation of olive oil produced with a new enzyme processing aid. *Journal of American Oil Chemist's Society, 74*, 1105–1113.

Ranalli, A., De Mattia, G., & Ferrante, M. L. (1998). The characteristics of percolate olive oils produced with a new processing aid. International. *Journal of Food Science and Technology, 32*, 289–297.

Ranalli, A., & Ferrante, M. L. (1996). Physico-chemical and analytical characteristics of extra-virgin olive oil extracted by using a pectolytic enzymatic processing aid. *Olivae, 9*, 27–32.

Ranalli, A., Gomes, T., Delcurator, D., Contento, S., & Lucera, L. (2003). Improving virgin olive oil quality by means of innovative extracting Biotechnologies. *Journal Agriculture and Food Chemistry, 51*, 2597–2607.

Ranalli, A., & Lazzari, M. (1996). New technological solutions for optimisation of olive oil quantity. *Riv. Ind. Chim., 34*, 74–84.

Ranalli, A., & Serraiocco, A. (1996). Quantitative and qualitative effect of a pectolytic enzyme in olive oil production. *Grasas Aceites, 47*, 227–236.

Ranalli, A., Sgaramella, A., & Surricchio, G. (1999). The new "Cytolase 0" enzyme processing aid improves quality and yields of virgin olive oil. *Food Chemistry, 66*, 443–454.

Servili, M., Baldioli, M., Selvaggini, R., Miniati, E., Macchioni, A., & Montedoro, G. F. (1999). High – performance liquid chromatography evaluation of phenols in olive fruit, virgin olive oil, vegetation waters, and pomace and 1D- and 2D-nuclear magnetic resonance characterization. *Journal of American Oil Chemist's Society, 76*, 873–882.

Vazquez Roncero, A., Janer del Valle, C., & Janer del Valle, M. L. (1973). *Grasas Aceites (Seville), 24*, 350.

Vierhuis, E., Korver, M., Schols, H. A., & Voragen, A. G. J. (2003). Structural characteristics of pectic polysacharides from olive fruit (*Olea europaea* cv moraiolo) in relation to processing for oil extraction. *Carbohydrate Polymers, 51*, 135–148.

Vierhuis, E., Servill, M., Baldioli, M., Schols, H. A., Voragen, A. G. J., & Montedoro, G. F. (2001). Effect of enzyme treatment during mechanical extraction of olive oil on phenolic compounds and polysaccharides. *Journal of Agriculture and Food Chemistry, 49*, 1218–1223.