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Aqueous extraction of virgin olive oil using industrial enzymes

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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 $3 \times 2 \times 3$ 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).

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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 ($^{*}p < 0.05$; $^{**}p < 0.01$).