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Research Paper

Improvement of Olive Oil Quality of Moroccan Picholine by *Bacillus Licheniformis* Enzyme's Preparation

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Abstract: *The present work deals with the development of an enzymatic treatment aiming at producing high-quality olive oil with amelioration of quality's parameters. The enzymatic preparation used contains principally cellulase and pectinase. The enzymatic preparation was added to the olive paste (Moroccan picholine) at the beginning of the malaxation step. Oil quality issued of extraction by enzymatic preparation had a higher quality. Phenolics content and antioxidant activity were increased significantly. The difference between the acidity, K232 and K270 indices shows clearly the positive effect of using enzymatic preparation on olive oil extraction.*

Keywords: Olive oil, Moroccan Picholine, oil quality, enzymatic extract.

Introduction

Olive oil, a natural product containing the wide range of bioactive compounds from olive fruit, is a well-known key component of the traditional Mediterranean diet, because of its high levels of typical phenolics and unsaturated fatty acids, which are believed to be associated with a relatively long life in good health [1]. The olive oil industry is very important in Mediterranean countries, both in terms of wealth and tradition. In particular, during extraction, the content of some components is significantly modified, depending on technique employed [2-3], and also new components can be formed as a result of chemical and/or enzymatic pathways [4].

In particular, during extraction, the content of some components is significantly modified, depending on the extraction technique employed [2-3], and also new components can be formed as a result of chemical and/or enzymatic pathways [4]. Many factors, such as malaxation temperature, time of

exposure of olive paste to air contact, use of microorganisms [5-6] or enzymes [7], among others, may significantly influence the extraction efficacy.

So, the optimisation of olive processing techniques cancontribute significantly to the improvement of the quality level of virgin olive oil [8].

Several authors reported that the addition of commercial enzyme preparations during malaxation can reduce the complexation of hydrophilic phenols with polysaccharides, increasing the concentration of free phenols in the olive paste and their consequent release into the oil and waste waters during processing [7-9, 10].

The aim of this work was to optimise olive oil extraction technology in terms of product characteristics, using an enzymatic preparation obtained by culture of *Bacillus lichenoformis,* during the malaxation of the Morocan picholine cultivar olive paste.

Materials and Methods

2.1. Olive Variety and Characteristics

The olives (*Olea europaea L.*) of the Moroccan Picholine variety produced in Fez – Morocco during 2008 and 2009, were collected and used for extraction experiments.

The percentage of olive moisture was determined by drying 10g of the olive paste at 105°C to constant weight, as described by Ranalli [4]. The residue was used for determination of the oil percentage which was carried out by a Soxhlet apparatus and petroleum ether (b.p. 40–60 ◦C) as solvent. The extract was dried at 80 °C and weighted. The olive paste solid content was evaluated as the difference between the total and oil plus moisture weights and expressed as percentage.

2.2. Enzyme Preparation and Experimental Process

B. licheniformis was isolated from a Moroccan soil and taxonomically identified by 16S rRNA gene sequencing. *B. licheniformis* was cultured for three day at 37°C in (NH4)2SO4, 1.4g/l; MgSO4, 0.3g/l; KH2PO4, 2g/l; CaCl2, 0.3g/l; NaNO3, 5g/l; and 1ml of trace element solution ((g/l) CoCl2: 2; MnSO4·H2O: 1.6; ZnSO4·H2O: 1.4; FeSO4·7H2O: 0.5) as described by Mandels [11]. The cells were centrifuged and supernatants were then filtrated using a 0.2µm millipore membrane.

The enzymatic solution contains enzymes produced by *B.licheniformis* specially polygalacturonase (41 IU/ml) and carboxymethyl cellulase (21 UI/ml). One unit of enzyme activity was defined as the amount of enzyme that produces 1mmol of galacturonic acid/min or glucose/min, under the assay conditions.

After manually stoning, the olive paste (about 100 g) was prepared using a food blender. Malaxation of paste was carried out in a mixer at 10 rpm for 1h. The enzyme preparation of was added to the paste at the beginning of the kneading step. This step was carried out at 20°C, 30 ◦C and 40°C. Separation of oily must from the paste and oil from the oily must was performed by centrifugation (6000rpm for 10min).

2.3. Oil Sample Analyses

The titratable acidity, the peroxide index and the determination of UV spectrophotometric indices (K232, K270 and ΔK) were determined from the extracted oils according to the European Union standard methods (Regulation EEC 2568/1991; Regulation CE 1989/2003). The K232 value is an indication of conjugation of polyunsaturated fatty acids in olive oils, whereas K270 is an indication of carbonylic compounds (aldehyde and ketones) [12].

Free acidity, given as % of oleic acid, was determined by titration of a solution of oil dissolved in ethanol– ether (1:1) with ethanolic potash.Peroxide index, expressed in milliequivalents of active oxygen per kilogram of oil (mEq O2/ kg), was determined as follows: a mixture of oil and chloroformacetic acid was left to react with a solution of potassium iodide in darkness; the free iodine was then titrated with a sodium thiosulfate solution. The pigments (carotenes and chlorophylls) were determined as described by Minguez-Mosquera [13]. In brief, 7.5 g of oil was weighted, dissolved in cyclohexane and taken to a final volume of 25 ml. The carotene and chlorophyll pigments were determined by measuring the absorbance at 470 and 670 nm, respectively. Results were expressed as mg of pheophytin ‘‘a’’ and lutein per kg of oil, respectively.

Statistical Analysis

All tests were carried out in triplicate. Statistical analysis was performed using ANOVA. Significant differences between results were determined at $p<0.05$, according to Duncan’s Multiple Range Test.

Results and Discussion

This work was performed with the Moroccan Picholine olives. Our results showed that this variety had a percentage of olive moisture of 49%, an oil percentage of 18% and a percentage of solid content of 33 % (Table 1).

Effect of the Enzymatic Solution on TP and OD

To evaluate the quality of the oils obtained by enzymatic extraction the quality standards by EEC 2003 norm have been considered. This norm refers to olive oils and pomace olive oils, and the corresponding analytical methods.

The effect of malaxation temperature on phenol profile of olive oils has been reported by caring out tree control tests at 20, 30 and 40 ◦C to select the value of this parameter at which both TP (Fig.1) and OD (Fig. 2) levels in the olive oil of the Moroccan picholine cultivar are maximized.

Our finding demonstrates clearly that the use of the enzymatic solution gave rise to greater amount of both TP and OD, if compared with control samples (Fig 1 and 2). Vierhuis [14] 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 enzymatic extract 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 [15-7].

This rise was observed at different temperature of malaxation. However, a temperature of 40°C marks a notable decrease in both of TP and OD. Danilo [16] has found that raise of this parameter from 20 to 30 ◦C resulted in an increased phenolics content, probably due to an enhanced release of oil constituents from the vegetable tissue [17]. Many previous works had reported the negative effect of hight temperature on olive oil phenolics content, sensory and healthy characteristics of olive oil [18-20, 21].

In particular, the loss of olive oil phenol compounds was mainly ascribed to the fact that the increased temperature improves the oxidation of these compounds due to the polyphenol oxidase and peroxidase activities [20]. In another work, Danilo [18] reported that enzymatic treatment yields a product with a potential higher resistance to oxidation and, consequently, with a potential longer shelf-life when compared to standard oils.

Effect of the Enzymatic Solution on Acidity

According to the statistical analysis depicted in Table 2, it can be concluded that there is a significant difference between the quality parameters corresponding to oils obtained the enzymatic solution in extraction and the control. All the oils produced and analyzed showed very low values for the regulated physicochemical analytical parameters evaluated, with all of them falling within the 'extra virgin' category, as stated by Regulation EC/1989/2003 [19]. Thus, all the oils can be classified as ''extra virgin olive oil" according to European regulations.

As regard to the quality parameters of the oils (Fig 3), the acidity is inferior when the olive fruits were extracted in presence of enzymatic preparation. In an other studies [22] using calcium carbonate in oil extraction doesn't have any improvement in acidity of oil.

These results agree with those reported by Cert et al. using talc as coadjuvant, and by Cruz et al. using common salt, so it can be concluded that these three coadjuvants produce just a physical action on oil extraction. We concluded that the use of the enzymatic solution produce an improvement of quality of the oil extracted.

Effect of the Enzymatic Solution on Peroxide Index

All tests showed a peroxide value of less than 20 meq O_2/kg: maximum limit for extra virgin olive oil [19]. These low values of peroxide index show that the oil was quickly extracted after olive harvest and was stored in good conditions. It suggests that the oil will not oxidize prematurely and will retain over time.

The lowest peroxide value was observed during the month of November in the extraction of olive oil Moroccan Picholine variety with the addition of enzyme extract during the mixing phase (Fig 4). Note that the peroxide index increases with the maturity of olives, the harvesting of December (ripe fruits) presented the highest peroxide index. Consistent results were obtained by Tanouti [23].

Effect of the Enzymatic Solution on UV Spectrophotometric Indices (K232, K270 and ΔK)

The value of peroxide index values ≤ 20meq O2/kg oil do not always mean the absence of oxidation. The using of the coefficients of determination (K232, K270) with ultraviolet absorbance may gives information about the presence or absence of secondary oxidation products in oil.

According to the results shown in Figure 5, we see that there is a significant difference between the refractive indices of olive oil obtained by addition of the enzymatic solution and the control. The K232, K270 and Delta K spectrophotometric values recorded during the various manipulations ranged from the maximum limits for virgin olive oil and extra virgin olive oil (Fig 5). All values obtained using the enzyme extract in step of kneading have therefore met the standards set by the IOC ($K232 \leq 2.5$, $K270 \leq 0.25$, Delta $K \leq 0.01$).

Olive oil from obtained from the harvest of December is characterized by a value of K270 (0.37) greater than 0.25, witch indicates that this oil contains second oxidation products (ketones, aldehydes ...); Unlike the oil extracted in the same harvest period (December) with the addition of enzyme extract during the kneading phase whose coefficient K270 does not exceed 0.22.

The oil extracted in the month of December, although in the standards, has also presented the highest average value of peroxide index (10 meq O_2/kg for testing and 10 meq O_2/kg for the control). These results may be related to several factors such as late harvest olives, excessive exposure of olives and the oil extracted oxygen from air and light.

Tables and Figures:

Table 1: Compositional characteristics of the Moroccan Picholine variety

Olive variety	Moisture (%)*	Oil (%)*	Solids (%)*
Moroccan Picholine	49 ± 0.05	18 ± 0.03	33 ± 0.03

Data are means of three replicates: coefficient of variance in all cases p <0.05%.

Table 1 shows composition: moisture (%), oil (%) and solids (%) of the Moroccan picholine used in this study.

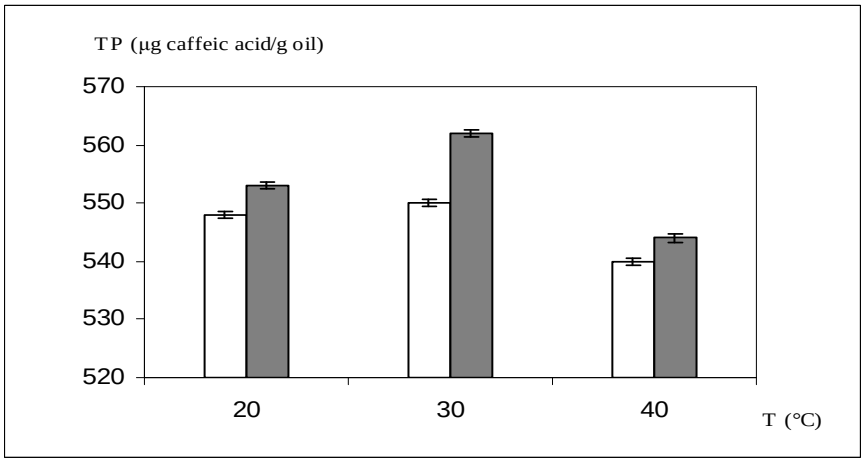


Fig. 1: Effect of the malaxation temperature and enzymatic preparation on TP

Figure 1 represents the effect of different malaxation temperatures on TP contents on olive oils obtained from stoned olive paste of *Morrocan picholine*.

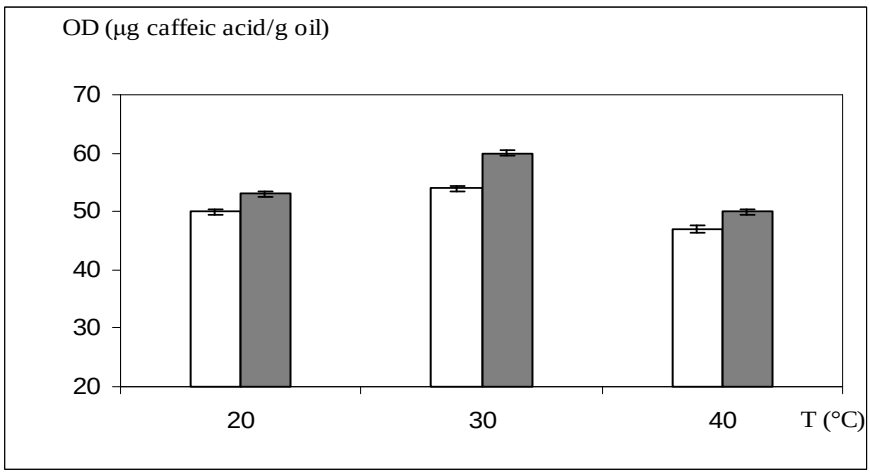


Fig. 2: Effect of the malaxation temperature and enzymatic preparation on OD contents

Figure 2 represents the effect of different malaxation temperatures on OD contents on olive oils obtained from stoned olive paste of *Morrocan picholine*.

Table 2: Effect of enzymatic treatment on quality parameters of olive oil

	K270	**K232**	**ΔK**	**% acidity**	**Peroxide value**
Control	0.25	2.55	0.0006	0.35	10.4
Test	0.18	1.85	0.0004	0.24	9.5
EEC 2003	≤ 0.22	≤2.5	≤0.01	≤0.8	≤20

Values are the means of five replicates.

Table 2 shows the effect of addition of enzymatic treatment to the olive paste of the Moroccan picholine compared to the control and the value of the EEC 2003 on % acidity, Peroxide value and UV spectrophotometric indices (K232, K270 and ΔK).

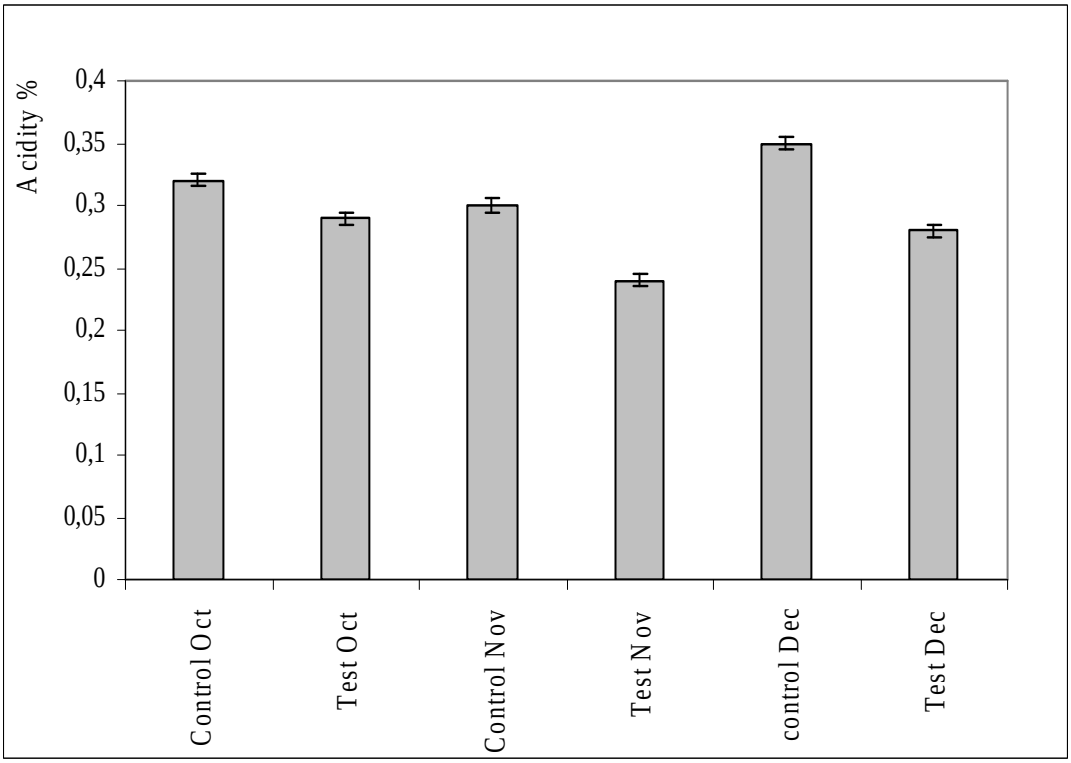


Fig. 3: Acidity % of olive oil from the Moroccan picholine compared to the controls

Figure 3 represents the percentage of acidity of the olive oil from the Moroccan picholine extracted with enzymatic solution from *B.lichenoformis.*

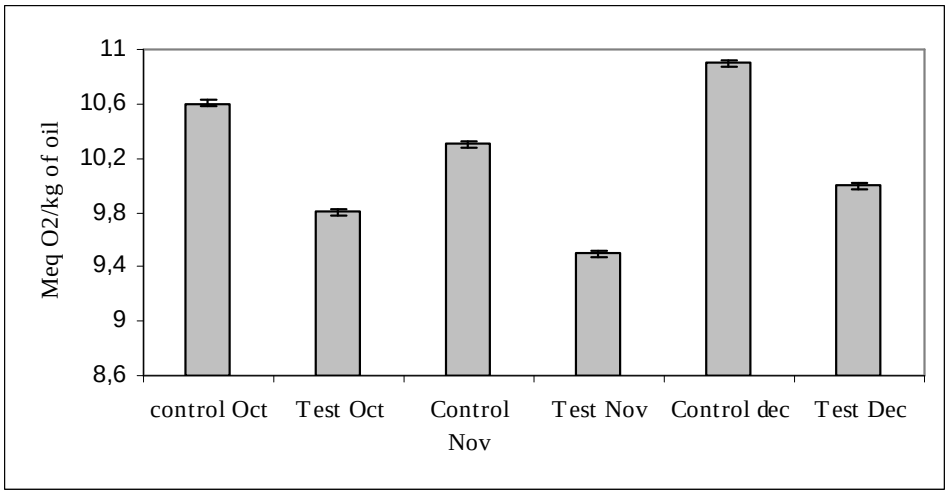
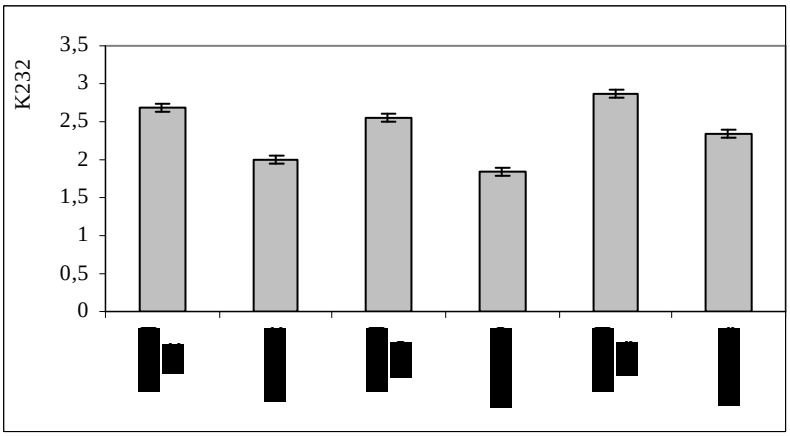
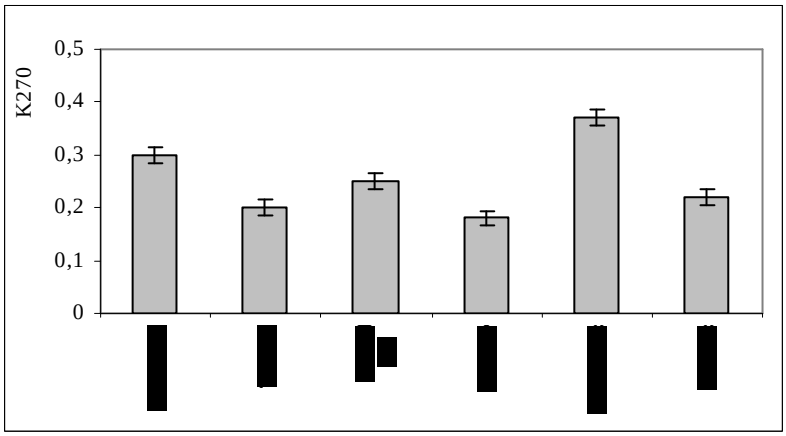


Fig. 4: Peroxide index of olive oil of morrocan picholine compared to the controls

Figure 4 represents the Peroxide index (meq O_2/Kg of oil) of the olive oil from the Moroccan picholine extracted with enzymatic solution from *B.lichenoformis.*





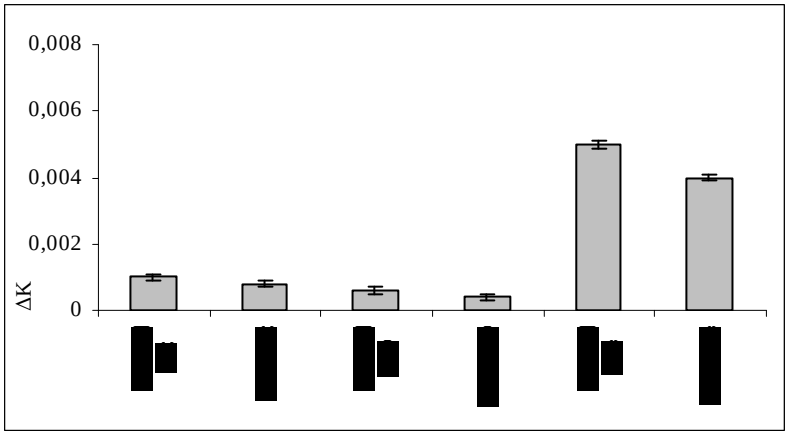


Fig. 5: Spectrophotometric indices (K232, K270 and ΔK) of olive oil from the Moroccan picholine compared to the controls

Figure 5 represents the value of Spectrophotometric indices (K232, K270 and ΔK) of the olive oil from the Moroccan picholine extracted with enzymatic solution from *B.lichenoformis.*

References

[1] F. Visioli and C. Galli, Olive oil phenols and their potential effects on human health, *J Agric Food Chem*, 46(1998), 4292-4296.

[2] M.T. Morales, F. Angerosa and R. Aparicio, Effect of the extraction conditions of virgin olive oil on the lipoxygenase cascade: Chemical and sensory implications, *Grasas Aceites*, 50(1999), 114-121.

[3] R. Amirante, E. Cini, G.L. Montel and A. Pasqualone, Influence of mixing and extraction parameters on virgin olive oil quality, *Grasas Aceites*, 52(2001), 198-201.

[4] A. Ranalli, M.L. Ferrante, G. De Mattia and N. Costantini, Analytical evaluation of virgin olive oil of first and second extraction, *J Agric Food Chem*, 47(1999), 417-424.

[5] F. Kachouri and M. Hamdi, Enhancement of polyphenols in olive oil by contact with fermented olive mill wastewater by *Lactobacillus plantarum*, *Process Biochem*, 39(2004), 841-845.

[6] F. Kachouri and M. Hamdi, Use *Lactobacillus plantarum* in olive oil process and improvement of phenolic compounds content, *J Food Eng*, 77(3) (2006), 746-752.

[7] E. Vierhuis, M. Servili, M. Baldioli, H.A. Schols, A.G.J. Voragen and G.F. Montedoro, Effect of enzyme treatment during mechanical extraction of olive oil on phenolic compounds and polysaccharides, *J Agric Food Chem*, 49(2001), 1218-1223.

[8] D. Catalano and F. Caponio, Machines for olive oil paste preparation producing quality virgin olive oil, *Fett/Lipid*, 98(1996), 408-412.

[9] A. Ranalli and G. Mattia, Characterisation of olive oil produced with a new enzyme processing aid, *J Am Oil Chem Soc*, 74(1997), 1105-1113.

[10] M. Mandels and J. Webers, The production of cellulases, *Advances in Chemistry Series: Amr Chernical Soc*, 95(1969), 3991-414.

[11] J.M. Garcia, F. Gutierrez, J.M. Castellano, S. Perdiguero and M.A. Albi, Influence of storage temperature on fruit ripening and olive oil quality, *J Agr Food Chem*, 44(1996), 264-267.

[12] M.I. Minguez-Mosquera, J.L. Rejano, B. Gandul, A. Higinio and J. Garrido, Colour pigment correlation in virgin olive oil, *J Am Oil Chem Soc*, 68(1991), 669-671.

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

[14] A. Ranalli, A. Sgaramella and G. Surricchio, The new ‘‘Cytolase 0” enzyme processing aid improves quality and yields of virgin olive oil, *Food Chem*, 66(1999), 443-454.

[15] D.F. Danilo, A. Bahar, A. Milvio, P. Patrizia and C. Attilio, Improvement of olive oil phenolics content by means of enzyme formulations: Effect of different enzyme activities and levels, *Biochem Eng J*, 41(2008), 149-156.

[16] A. Ranalli, S. Contento, C. Schiamone and N. Simone, Malaxing temperature affects volatile and phenol composition as well as other analytical features of virgin olive oil, *Eur J Lipid Sci Technol*, 103(2001), 228-238.

[17] F. Angerosa, R. Mostallino, C. Basti and R. Vito, Influence of malaxation temperature and time on the quality of virgin olive oils, *Food Chem*, 72(2001), 19-28.

[18] European Union Regulation EEC, Characteristics of olive and olive-pomace oils and on their analytical methods, Modify at Regulation EEC/1989/03, *Off J Eur Commun*, L295(2003), 57-77.

[19] M. Servili, R. Selvaggini, A. Taticchi, S. Esposto and G.F. Montedoro, Volatile compounds and phenolic composition of virgin olive oil: Optimisation of temperature and time of exposure of olive paste to air contact during the mechanical extraction, *J Agric Food Chem*, 51(2003), 7980-7988.

[20] J.J. Salas and J. S´anchez, The decrease of virgin olive oil flavor produced by high malaxation temperature is due to inactivation of hydroperoxide lyase, *J Agric Food Chem*, 47(1999), 809-812.

[21] F. Espinola, M. Manuel, G. Diego and C. Eulogio, Improved extraction of virgin olive oil using calcium carbonate as coadjuvant extractant, *J Food Eng*, 92(2009), 112-118.

[22] K. Tanouti, H.S. Caid, M. Abid, A. Mihamou, M. Khiar, M.E. Hachem, Y. Bahetta and A. Elamrani, Isly Huile d'Olive Vierge Analyse des Triglycérides et Composition en Acides Gras (Volume N° 23), Les Techniques de Laboratoire, 2011.