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Improving Olive Oil Yield from Moroccan Picholine by Bacterial enzymes extract

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ABSTRACT

Olive oil production represents one of the most interesting fields of Moroccan agriculture. The effect of the Bacterial enzyme preparations during the mechanical extraction process of virgin olive oil on the yield was investigated. In this sense, we implicated the usage of Bacillus licheniformis enzymatic solution (containing among other enzymes cellulase and pectinase) on improving and maximising the oil yield during olive mechanical extraction. The assays were performed with the Moroccan Picholine variety. Results indicated that Bacillus licheniformis enzymatic solution increased olive oil extraction yield (4.12g/100 of olives) compared to control (3.79g/100g of olives). Moreover, the use of commercial cellulase or pectinase for oil extraction doesn't give more olive oil than the bacterial enzymes solution (4g/100g of olives and 4.05g/100g of olives respectively). We have also shown that olives should be used during ripening stage from the middle of November in order to obtain the highest olive oil yield.

Key words: Extraction, Olive, Oil Yield, Enzymes, Moroccan Picholine, Ripening Stage.

INTRODUCTION

Virgin Olive oil, the major edible vegetable oil of the Mediterranean countries, is obtained from the olive fruit *Olea europaea L*. The mechanical extraction process include washing; crushing, grinding, pressing; and decanting or centrifugating (EC Regulation 2001) under conditions, particularly thermal, that do not alter the quality of the oil (IOOC 2003). While all types of olive oil are sources of monounsaturated fat acid, extra virgin olive oil from the first pressing contains higher levels of antioxidants, particularly vitamin E and phenols (Visioli and Galli 1998). This is why developing methods that increase the extra virgin oil yield is highly investigated (Chiacchierini et al 2007).

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The olive fruit contains about 50 % of water, 20 % of oil and 20 % of carbohydrates (pectic, cellulosic and hemocellulosic substances), organic acids, pigments, phenolic compounds and minerals. Most of the oil (96-98 %) is found in the flesh (mesocarp) and the skin (pericarp) and only 2-4 % is found in the pit (endocarp). Not all the oil present in the olives is released: some remains inside the unsheltered cells, some is left in the colloidal system of the olive paste (microgels) and some is bound in an emulsion with the vegetable water (Francisco et al. 2008). The difficulty of freeing this ‘‘bound” oil lies mainly in the fact that the droplets of dispersed or emulsified oil are surrounded by a lipoprotein membrane (phospholipids and proteins) that keeps them imprisoned (Boskou 1996; Petursson et al. 2004). In order to effectively recover the olive oil enclosed in the cells, the polysaccharides, which make the cell wall structures, must be destroyed by enzymatic digestion.

In this study, we tested the effect of the *Bacillus licheniformis* enzymatic solution that contains, among other enzymes: cellulase and pectinase on olive oil extraction of the Moroccan Picholine cultivar. The purpose of this work was to study the influence of the application of *Bacillus licheniformis* enzymes on olive oil yield.

MATERIALS AND METHODS

Materials:

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 (1999).

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.

Bacterial culture and enzymes preparation from supernatants :

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 (1976). The cells were centrifuged and supernatants were then filtrated

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using a 0.2µm millipore membrane. The enzymatic solution contains enzymes produced by B.licheniformis specially polygalacturonase (41 IU/ml) and carboxymethyl cellulase (21 U/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.

Olive Oil processing and extraction :

After the crushing, 100 g of olive paste was prepared using a food blender. The bacterial supernatant containing enzyme was added to the paste at the beginning of the kneading step. Then, the preparation is incubated at a giving temperature for 45 min. Separation of the oil from the paste was obtained by a centrifugation at 4500g for 30 min.

Effect of kneading temperature, commercial enzymes and state of maturity of olives on oil yield :

The test of enzymatic treatment to enhance the oil yield was evaluated by adding commercial enzymes cellulase and pectinase from Aspergillus niger (Sigma) to the olives paste during kneading. The best enzyme concentration was determined on the basis of increase in oil yield (%) compared to control as indicated. The controls were carried out using water instead of enzyme solution.

Furthermore, five different temperatures, 30, 37, 40, 45 and 50□C, were tested to determine the optimum conditions for the production of olive oil by the supernatants containing enzymes produced by B. licheniformis. The maturity state of the fruit on olive oil yield, from October to December during two consecutive years of harvest 2008 and 2009, was also evaluated.

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

Effect of B. licheniformis enzymatic solution on olive oil yield:

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). The extraction yield of olive oil from this Moroccan Picholine variety showed an improved: 4.12g of oil /100g of olives by addition of the

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enzymatic solution of B. licheniformis. It seems that the enzymatic treatment degrades the walls of the oil-bearing cells that elude to a crushing (Coimbra et al. 1994) and also has similar effects on the colloidal system in olive paste (pectins, hemicelluloses, proteins, etc.) that retain the droplets of oil (Ranalli et al. 1997; Voragen et al. 2001).

Effect of B. licheniformis enzymatic solution and temperature on olive oil yield:

The effect of B. licheniformis enzymatic solution and temperature on the oil yield from the olive of the Moroccan Picholine cultivar was investigated. Five tests were carried out at 30, 37, 40, 45 and 50□C to select the value of this parameter for which the oil yield is maximised. As showed in Figure 1, incubation of the olive paste with B. licheniformis enzymatic solution at tested temperature of 37°C lead to maximum oil yield of 4.12g/100g of olives. The oil yield was 4, 3.86, 3.66 and 3.5g of oil /100g of olives obtained at 30, 40, 45 and 50°C respectively. The optimal treatment temperature has been reported to vary between 30°C by Danilo De Faveri (2008) and 45°C by Soto (2007).

So, to improve coalescence of the dispersed oil droplets, the paste temperature of the olive paste can be increased, thus reducing viscosity. In aggregate, our data suggest that combination synergitic, or optimised conditions are obtained at 37°C in the presence of B. licheniformis enzymatic solution. However, the amount of recovered oil decreased at higher temperatures. This effect is similar to that obtained by Concha (2004) for rosehip oil and by Zuniga (2003) for chilean hazelnut oil extraction. Thermal inactivation of enzymes might explain this observed effect. Enzymes are intrinsically labile, but temperature could lead to opposite effects on their stability and reactivity, which becomes an important variable in any process that involves biocatalysis (Illanes 1999). Another explanation could be the decreasing of soluble sugar production by enzyme hydrolysis at higher temperatures. The enzymes degrade the oilseed cell wall by converting the cellulose materials into glucose (Visioli et al. 1998). So, these sugars could be caramelized and limiting the oil release from the cells (Zuniga et al. 2001). We opted to select the kneading temperature of 37°C as the optimal temperature to perform the subsequent tests.

Effect of Commercial enzymes on olive oil yield:

The use of commercial cellulase or pectinase (Sigma) allowed a marked increase of 4 and 4.05g of oil/100g of olives respectively, as compared to control (Fig.2). The pectinase enzyme gave a greater yield. This can be explained by the fact that pectic substances are more abundant in cell wall of olives. However, the combination of the two enzymes, cellulase and pectinase, resulted in a superior increase which reaches 4.5g of oil/100g of olives.

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Previous work has shown variable yields of extractions of olive oil. The maximum yield of olive oil extraction was 17.5 % using three enzymes formulation: Uvazym Extra, Maxoliva and Uvazym Couleur in the proportions (1/3, 1/3, 1/3) (v/v/v) (Aliakbarian et al. 2008). Other studies have shown that the values of olive oil yield recorded for various enzymatic treatments ranged as follows: 9.14% for pectinase , 9.29% for cellulase and 9.75% for pectinase + cellulase (Sharma et al. 2007). Systematic studies carried out in the 1980s, revealed that no single enzyme was adequate for the efficient maceration and extraction of oil from olives. Combination of enzymes yields more oil; witch might be due to their combined effect on colloidal and lipoproteic structures of olive fruits (Ranalli et al. 1997).

However, the use of Bacillus licheniformis enzymatic solution resulted in an increase exceeding that obtained using, cellulase or pectinase, commercial but not both simultaneously. On the other hand, commercial enzyme preparations, such as Olivex (a pectinase preparation with low levels of cellulase and hemicellulase from Aspergillus aculeatus) (Vierhuis et al. 2001) and Cytolase 0 (Ranalli et al. 1999), were successfully used to enhance the olive oil yield.

We added different concentration of enzymes used: 0.01%, 0.025%. 0.05%, 0.1% and 0.15% (Fig.3). Based on our results, the increasing enzyme concentration increased olive oil yield. The lowest enzyme concentration (0.01%) gave a small increase for the production of oil which doesn't exceed 3.5g of oil /100g of olives. The optimal production has been observed after the use of the enzyme concentration 0.1% which gave an increase of 4.1g of oil /100g .In excess of this concentration, we observed saturation. So any increase in enzyme levels can be considered as a loss. Najafian (2009) have shown that Pectinex Ultra SP-L achieve higher yields using 0.02% (v/w) of enzyme.

Effect of the ripening stage of olive on oil yield:

We studied the effect of ripening stage of olive on the oil yield during two campaigns 2008 and 2009. Oil yield was significantly affected by the ripening stage of the fruit. In early of October, Lipogenesis phase, the content of oil in olives was very low, as reflected in the low maximum oil yield of about 3.5g/100g of olives (Fig.4). Later, during the last week of October, an important increase of olive oil yield (4.05g/100g of olives) is obtained. Interestingly, the highest oil yield of 4.12g/100g of oil was reached in the first week of November (Oil accumulation phase), followed by a decrease 3.72g/100g of olives in

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December (Final phase of ripening) (fig 4). So, the first week of November appears to be the optimal period for harvesting the olives of the Moroccan Picholine variety.

These results are coherent with those obtained by Beltran (2004) who confirm that olives of the most important Spanish and Italian cultivars, 'Picual', 'Hojiblanca' and 'Frantoio' should be carried out from the middle of November in order to obtain the highest oil yield. However, other work (Koutsaftakis et al. 1999) shown that the optimal period for harvesting the olives was December.

Our results are consistent with the growth curve of olives and oil accumulation described by different authors (Hartmann 1949; Schulman et al. 1979) which shows that the growth curve of the olive fruit represents five different phases of growth, lipogenesis begins only at the end of the third phase of growth (Fig.4). In early October the fruit is still under development and begins lipogenesis. Then, the fruit reaches its maximum growth phase which is parallel to the maximum rate of oil accumulation (Lavee 1977). At the end of its development, December, the fruit loses water and loses its rigidity; the extraction of oil becomes easier mechanically, and enzymes have not much substrate to degrade, so increased yield is not important compared to the control.

Conclusion

This work has shown that enzymatic solution of B. licheniformis can be used in olive oil extraction. This enzymatic solution improves the yield of the olive oil from the Moroccan Picholine cultivar. In fact this yield is increased (4.12g of oil /100g of olives) at 37°C. Nevertheless, ripening stage of olive was a significant factor; the first week of November was the optimal period for harvesting the olives; the olive oil yield was maximized in this period. Studies are underway to determine the quality of olive oil by the B. licheniformis enzymatic solution in the same conditions.

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Table 1 Compositional characteristics of the Moroccan Picholine variety

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The table describes the composition of the Moroccan Picholine variety by percentage of moisture, oil and solids. Each experiment was performed three times, results with $p<0.05$ were considered statistically significant.

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 $p < 0.05\%$.

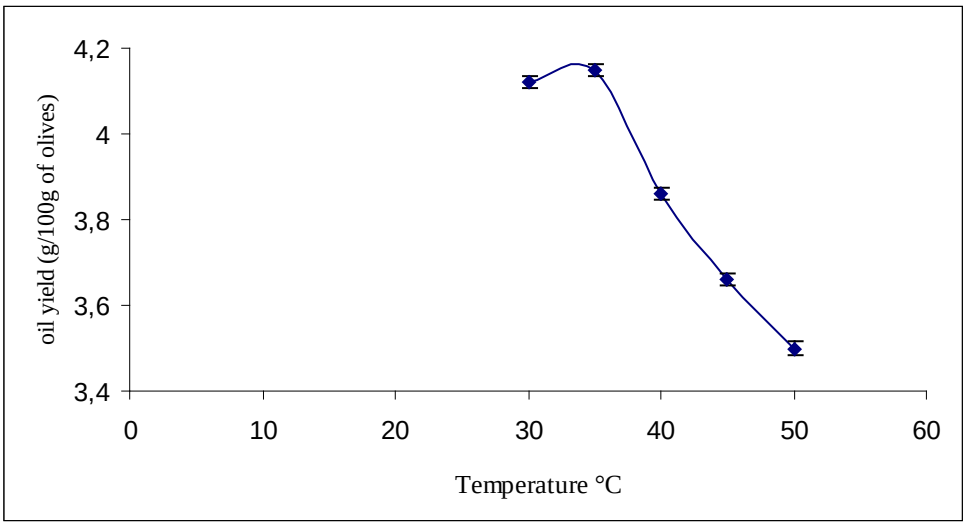

Fig.1 Effect of different kneading temperatures on oil yield % of olive paste of Moroccan Picholine variety.

The Fig.1 shows the oil yield (%) at different temperatures. Each temperature test was performed five times, and the data averaged (n = 5). Values of oil yield (%) are significantly different at the level of $p<0.05$ according to the Duncan's Multiple Range Test.

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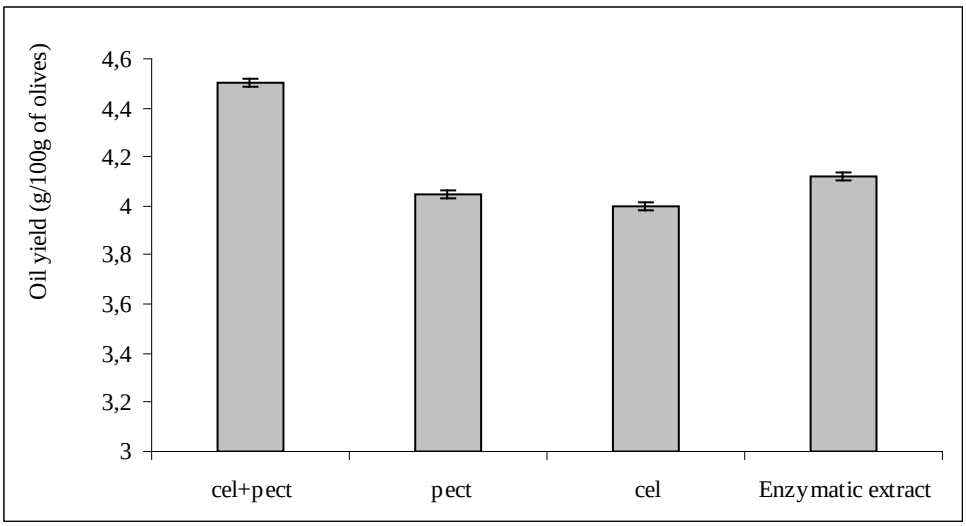

Fig.2 Effect of different commercial enzyme formulations (Cel, Pect and Cel+Pect) on oil yield (g/100g of olives) of olive paste of Moroccan Picholine variety.

The Fig.2 shows the oil yield (%) using commercial cellulase, commercial pectinase and a combination of both of them compared to the enzymatic solution of B.licheniformis. Each test was performed three times, and the data averaged (n = 3). Values of oil yield (%) are significantly different at the level of $p<0.05$ according to the Duncan's Multiple Range Test.

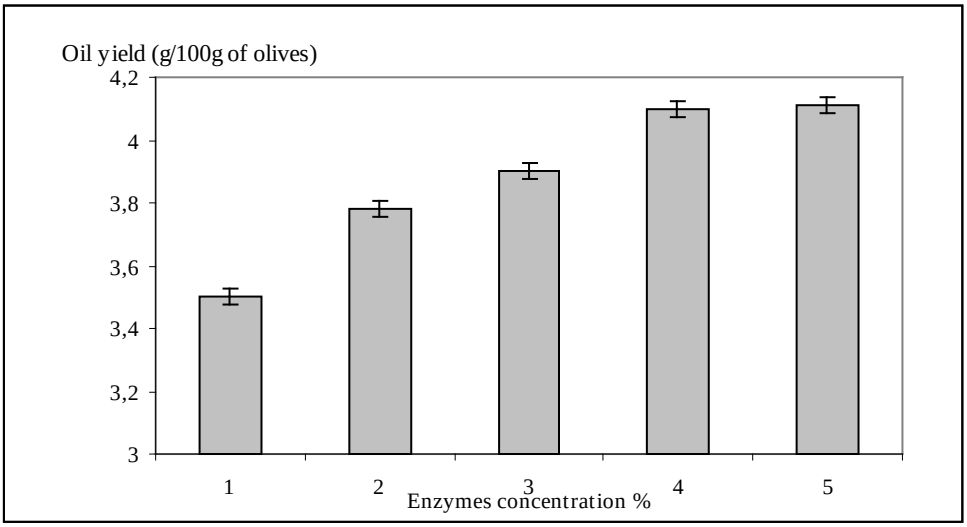

Fig.3 Effect of enzymes concentration on oil yield % of olive paste of Moroccan Picholine variety. Enzyme concentrations %: 1: 0.01; 2: 0.025; 3: 0.05; 4: 0.1, 5: 0.15

The Fig.3 describes the oil yield (%) using different concentrations of commercial cellulose and pectinase. Each test was performed three times, and the data averaged (n = 3). Values of

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oil yield (%) are significantly different at the level of $p<0.05$ according to the Duncan's Multiple Range Test.

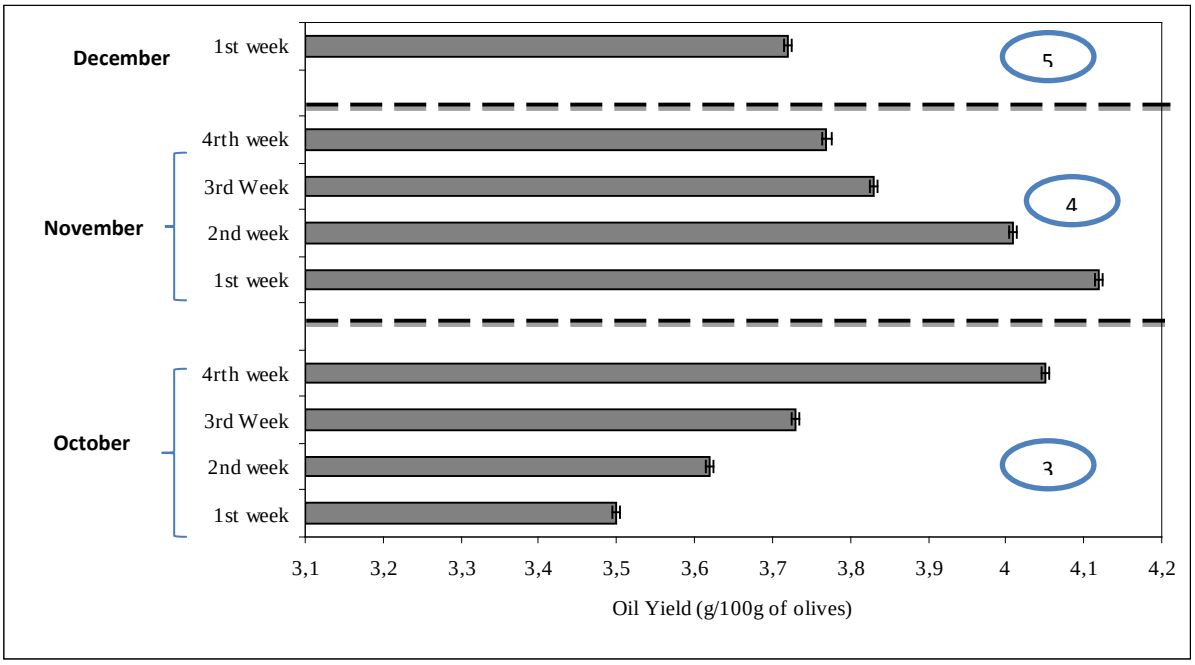

Fig.4 Effect of stage of ripeness of olives on oil yield % of Moroccan Picholine variety

The Fig.4 illustrates the variation of oil yield (%) according to the ripening stage of olive.

③ Lipogenesis phase ④ Oil accumulation phase ⑤ Final phase of ripening

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