Bio-extraction of olive oil: improvement of quality and extraction outputs

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This work focuses on the development of a bio-extraction process by means of enzyme formulations, added at the beginning of the malaxation step, aiming at producing olive oils of *Coratina* cultivar with increased quality characteristics and extraction outputs. In particular, a combination of a 3^2 full factorial design with response surface methodology was used to investigate the combined effects of the malaxation time (t) and the concentration of the enzyme formulation (X). The concentrations of total polyphenols (*TP*) and *o*-diphenols (*OD*), antiradical power (*ARP*) and the oil extraction yield (Y) were selected as response variables. The optimum operating conditions predicted by the model (t = 92.00 min and X = 24.68 mL/kg_{paste}) assured the following responses: *TP* = 855.60 $\mu g_{CAE}/g_{oil}$, *OD* = 128.40 $\mu g_{CAE}/g_{oil}$, *ARP* = 25.28 $\mu g_{DPPH}/\mu L_{extract}$ and Y = 15.45 $g_{oil}/100g_{paste}$.

1. Introduction

The beneficial health effects of olive oil are due to both its high content of monounsaturated fatty acids and its high content of antioxidative substances (Visioli & Galli, 1998). 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 (Morales & Aparicio, 1999; Amirante et al., 2001), and also new components can be formed as a result of chemical and/or enzymatic pathways (Ranalli et al., 1999a). Many factors, such as malaxation temperature, time of exposure of olive paste to air contact (Servilli et al., 2003), use of microorganisms (Kachouri & Hamdi, 2004, 2006) or enzymes (Vierhuis et al., 2001), among others, may significantly influence the extraction efficacy.

Several authors reported that the addition of commercial enzyme preparations during malaxation degrade the walls of the oil bearing cells which retain the oil droplets and can also 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 (Ranalli & De Mattia, 1997; Vierhuis et al., 2001; De Faveri et al., under revision).

In order to face the increased competition of foreign products as well as to avoid the risk of marginalization and the loss of home and foreign market shares, the Italian olive oil sector has to concentrate on quality. So, with the aim of contributing to the increase in

the competitiveness of Italian olive oils, the present work focuses on the development of new technologies for its production studying the effect of an enzymatic treatment during olive paste malaxation on the phenolics content in olive oil of the *Coratina* cultivar using three different enzyme formulations at three different concentrations as well as their binary and ternary mixtures at a constant level and finally, to identify the most suitable operating conditions for the process, different tests were performed according to a 3^2 full factorial design, selecting the time of the malaxation process (*t*) and the concentration of the homogeneous ternary mixture of the enzyme formulations (*X*) as independent variables and total polyphenols concentration (*TP*), *o*-diphenols concentration (*OD*), antiradical power (*ARP*) and the oil extraction yield (*Y*) as response variables, and the collected results were worked out by RSM.

2. Materials and method

2.1 Enzyme preparation and experimental process

For the experimental extraction tests stoned olive paste of *Coratina* variety was used. Olive oil bio-extractions were carried out according to the procedure described by De Faveri et al. (under revision). The enzyme formulations (A, B and C) were added to the paste at the beginning of the malaxation step using three different levels (5, 10 and 15 mL/kg_{paste}) and their synergistic effects were also assessed by using their binary (A:B, A:C and B:C, 50:50%, v/v) and ternary (A:B:C, 33.3:33.3:33.3%, v/v/v) mixtures at a constant level (10 mL/kg_{paste}). Afterwards, the ternary mixture at three different levels and different malaxation times, according to the experimental design (see later), was used. In particular, A, B and C are complex formulations essentially containing pectinase plus cellulase and hemicellulase, pectinase and hemicellulase, and pectinase plus some minor activities, respectively.

2.2 Oil samples analyses

Phenolics were extracted from the oils according to the procedure described by Aliakbarian et al. (under revision). Total ploypheno1s (*TP*) and *o*-diphenols (*OD*) analyses, both expressed as $\mu g_{CAE}/g_{oil}$, were determined with Folin-Ciocalteau reagent and molybdate, respectively, according to Gutfinger (1981). Antiradical power were estimated using the DPPH assay in accordance with Brand-Williams et al. (1995) and was expressed as $\mu g_{DPPH}/mL$.

2.3 Experimental design

A 3^2 full-factorial experimental design was used to point out the relationship existing between the response functions and process variables as well as to determine those conditions able to optimise the extraction process. The two independent variables were coded according to the following equation:

$$x_i = \frac{\left(X_i - X_0\right)}{\Delta X_i} \qquad \text{i} = 1, 2 \qquad (1)$$

where x_i and X_i are the dimensionless and the actual values of the independent variable i, X_0 the actual value of the independent variable i at the central point, and ΔX_i the step

change of X_i corresponding to a unit variation of the dimensionless value. In order to simultaneously optimise the oil extraction process, the malaxation time (t, i = 1) in the range 60-120 min and the concentration of the ternary mixture (X, i = 2) in the range 5-25 mL/kg_{paste} were selected as the independent variables and *TP*, *OD*, *ARP* and *Y* ($g_{oil}/100g_{paste}$), were chosen as responses.
The behavior of the system can be described by the following second-order polynomial equation:

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \beta_{ij} x_i x_j \qquad (2)$$

where Y is the predicted response, β_0 is the interception coefficient, β_i are the linear terms, β_{ii} are the quadratic terms, β_{ij} are the interaction terms, and x_i and x_j represent the coded levels of the independent variables. The Student's t-test permitted us to check the statistical significance of the regression coefficients. The Fisher's test for analysis of variance (ANOVA) was performed on experimental data to evaluate the statistical significance of the model. The ''Statistica'' software (version 6.0, StatSoft, Tulsa, OK) and the ''Design Expert'' software (trial version 6.0.10, Stat-Ease, Minneapolis, MN) were employed for the regression analysis and the graphical optimization, respectively.

3. Results and discussion

3.1. Influence of malaxation temperature

Results illustrated in Fig. 1 demonstrate that a raise of malaxation temperature from 20 to 30°C resulted in an increased phenolics content, probably due to an enhanced release of oil constituents from vegetable tissue (Ranalli et al., 2001). A malaxation temperature higher than 30°C led to a decrease of these components in olive oil. Such a behaviour is in good agreement with a previous work which ascribed the increasing oxidation of phenolic contents of olive oil to the polyphenol oxidase and proxidase activities as a negative effect of high temperature (Servilli et al., 2003). Thus, malaxation temperature of 30°C was selected as the optimal temperature and the successive tests were performed at this temperature.

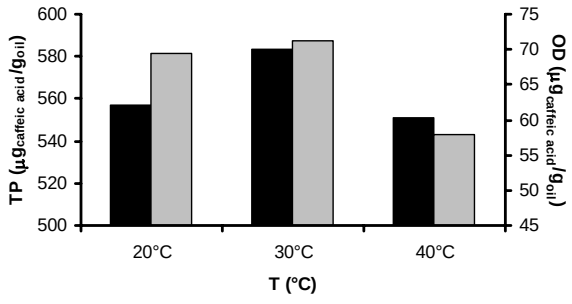

Fig.1 Effect of different malaxation temperatures on TP (■) and OD (■) contents in olive oils obtained from stoned olive paste of Coratina variety.

3.2. Effect of single, binary and ternary mixtures of enzyme formulations and levels

As illustrated in Fig. 2a and Fig. 2b, increasing the enzyme level from 5 up to 15 mL/kg_{paste} and working with formulation A, followed by formulations B and C, gave rise to greater amounts of both *TP* and *OD*. These results confirm the more marked effect of the enzymes when pectolytic was used in combination with both cellulase and hemicellulase activities (formulation A), followed by the combined effect of pectinase and hemicellulase activities (formulation B), which resulted in significantly increased phenolics contents in the oils if compared with control tests (without using enzyme formulations).

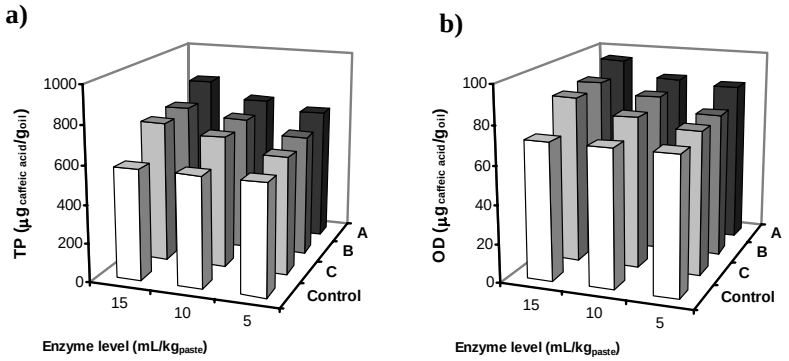

Fig.2 Effect of different enzyme formulations (A, B and C) and levels on TP (a) and OD (b) contents in olive oils obtained from stoned olive paste of Coratina variety.

In addition, four additional tests were performed using both binary and ternary mixtures of the three enzyme formulations. Table 1 lists *TP* and *OD* contents in olive oils treated with these formulations (A, B and C) at 10 mL/kg_{paste} or with binary and ternary mixtures at the same level.

Table 1
Effect of single, binary and ternary mixtures of enzyme formulations on *TP* and *OD* contents in olive oils obtained from stoned olive paste of *Coratina* variety compared with control test.

Enzyme formulation	*TP* ($\mu g_{CAE}/g_{oil}$)	*TP* increase (%)	*OD* ($\mu g_{CAE}/g_{oil}$)	*OD* increase (%)
A:B:C	804.3	40	97.69	37
A:B	786.3	37	87.77	23
A:C	723.3	26	83.15	17
B:C	690.9	20	82.20	15
A	740.6	29	86.17	21
B	705.6	23	82.75	16
C	686.4	19	78.77	10
Control	575.1	-	71.31	-

As expected, the effect of the ternary system (A:B:C) was more marked, followed by A:B, A:C and B:C, which put in evidence the importance of cellulase activity which was only contained in formulation A. Besides, results confirm the usefulness of pectinase, which are claimed to break down complex polysaccharides of plant tissue and are used in various biotechnological applications (Kashyap et al., 2001). For instance, similar commercial enzyme preparations, such as Olivex (Vierhuis et al., 2001) and Cytolase 0 (Ranalli et al., 1999b), were successfully used to enhance the release of phenolics in olive oil.

Our results point out the synergistic effect of different enzymatic activities in the extraction of phenolic compounds. Finally, since the best results were obtained when using the ternary mixture, such an enzymatic complex was selected to investigate the effect of enzyme processing aids as a function of malaxation time.

3.3. Statistics

3.3.1 Statistical modeling of TP, OD, ARP and Y according to the 3^2 full-factorial design

In order to optimise the oil extraction process, tests were carried out varying the malaxation time (*t*) in the range 60-120 min and the concentration of the ternary mixture (*X*) in the range 5-25 mL/kg_{paste} and *TP*, *OD*, *ARP* and *Y* were chosen as responses. Each test was performed in duplicate, while the central points were tested three times. The predicted models developed for *TP* (Y_1), *OD* (Y_2), *ARP* (Y_3), and *Y* (Y_4) are reported in Table 2.

Table 2
Response Surface Equations of *TP* (a), *OD* (b), *ARP* (c) and *Y* (d)

	Response function	Coefficient of Determination (R^2)
a)	$Y_1 = 717.30 - 12.05x_1 + 143.90x_2 - 43.82x_1^2$	0.968
b)	$Y_2 = 85.36 + 16.58x_1 + 28.37x_2 + 15.40x_2^2$	0.954
c)	$Y_3 = 23.80 - 0.79x_1 + 1.59x_2 - 2.26x_1^2$	0.789
d)	$Y_4 = 14.19 + 1.17x_1 + 1.21x_2$	0.760

Fig. 3 shows the three dimensional graphs obtained from the model calculated for the four responses. Both the best *TP* (Fig. 3a) and *ARP* (Fig. 3c) results were obtained at the highest *X* value (25 mL/kg_{paste}) and an intermediate *t* value (90 min), while a further increase in *OD* was found in the range 90-120 min malaxation time, irrespective of enzyme concentration (Fig. 3b). Finally, the response surface of the oil extraction yield (Fig. 3d) is represented by a plane, showing the maximum values at the highest level of both the independent variables.

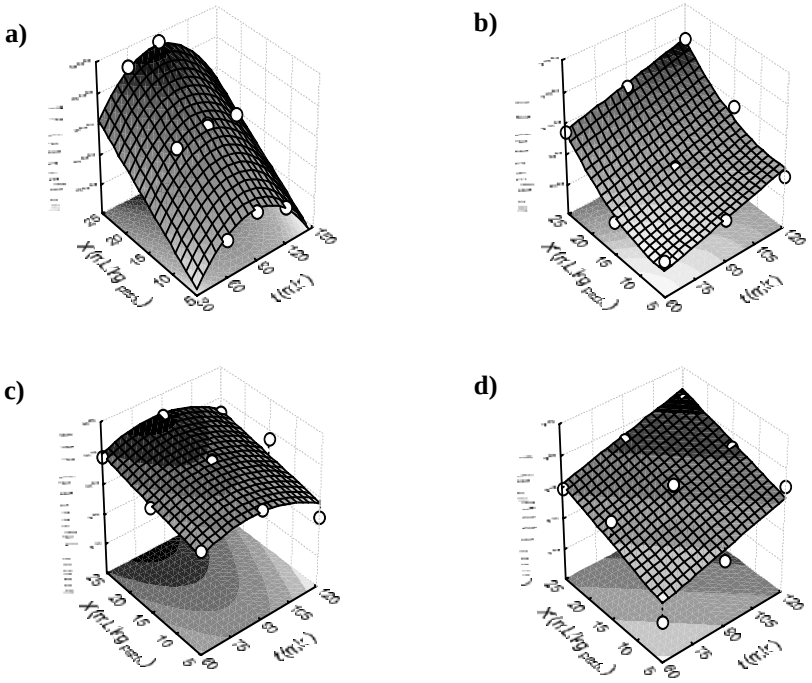

Fig.3 Response surfaces of TP (a), OD (b), ARP (c), and Y (d), as simultaneous function of (t) and (X) according to the 3^2 full-factorial design.

The regression analysis points out the significance of the linear *X* term (x_2) on each of the responses under investigation, while the quadratic term (x_2^2)of this independent variable was statistically significant only for Y_2. The linear term of *t* (x_1) was statistically significant for both Y_2 and Y_4, while it was kept in the other models (Y_1 and Y_3) to respect the hierarchical property. Moreover, no statistically significant interaction between the two independent variables ($x_1 \cdot x_2$) took place for all the four models under investigation.

3.3.2 Graphical optimisation

With the aim of definitely pointing out the optimal conditions of olive oil extraction by means of the proposed enzymatic treatment, a graphical optimisation was conducted using the "Design expert" software. Such a methodology essentially consists of overlaying the curves of the four models, obtained from the 3^2 full-factorial design, according to the specific criteria imposed.

The optimal working conditions were defined so as to get a high-quality olive oil with an increased phenolics level, in terms of both *TP* and *OD*, with a high *ARP*, and simultaneously maximizing the *Y*, to meet industrial economic requirements. Thus, the following constraints were imposed: $TP > 800$ $\mu g_{CAE}/g_{oil}$, $OD > 100$ $\mu g_{CAE}/g_{oil}$, $ARP > 24$ $\mu g_{DPPH}/\mu L_{extract}$ and $Y > 15$ $g_{oil}/100g_{paste}$.

Fig. 4 shows the overlay plot in which the white area represents the *t*-*X* dominion satisfying the imposed criteria. The point identified by the flag was chosen in the graph as representative of the optimised area corresponding to a malaxation time of 92.00 min and an enzyme concentration of 24.68 mL/kg_{paste}. Under these conditions the model predicted *TP* = 855.60 $\mu g_{CAE}/g_{oil}$, *OD* = 128.40 $\mu g_{CAE}/g_{oil}$, *ARP* = 25.28 $\mu g_{DPPH}/\mu L_{extract}$ and *Y* = 15.45 $g_{oil}/100g_{paste}$.

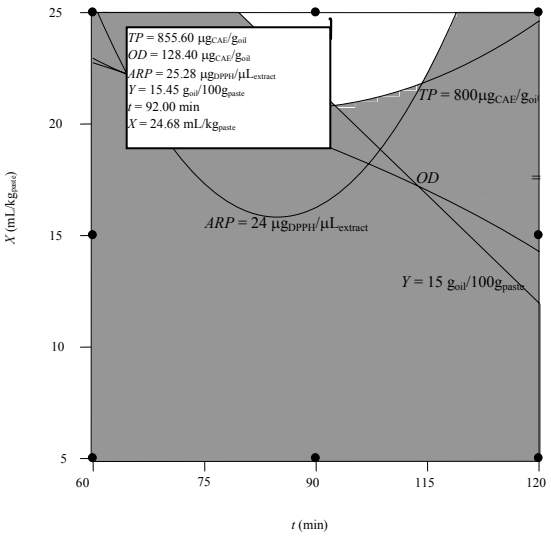

Fig. 4 Graphical optimisation identified by overlying plots of the four responses, TP, OD, ARP, and Y, as simultaneous functions of (t) and (X) according to the 3^2 full-factorial design.

4. Conclusion

A synergistic effect between the different enzymatic activities was observed and the ternary mixture was selected as the most efficient enzymatic system. The combined effects of malaxation time and the concentration of the selected enzyme formulation on the oil extraction process from olive fruits of the Italian cultivar *Coratina* were then studied. The highest levels of *TP* (874 $\mu g_{CAE}/g_{oil}$) and *ARP (*25.1 $\mu g_{DPPH}/\mu L_{extract}$) were reached at an intermediate malaxation time (*t* = 90 min) and the highest enzyme concentration (*X* = 25 mL/kg_{paste}), while the highest *OD (*147 $\mu g_{CAE}/g_{oil}$) was achieved at a malaxation time of 120 min and the same enzyme concentration. Moreover, the highest *Y* (16.0 $g_{oil}/100g_{paste}$) was reached at the highest malaxation time (t = 120 min), always at the highest enzyme concentration. The statistical model allowed identifying the optimum operating conditions (*t* = 92.00 min and *X* = 24.68 mL/kg_{paste}) able to simultaneously maximize *TP* (855.60 $\mu g_{CAE}/g_{oil}$), *OD* (128.40 $\mu g_{CAE}/g_{oil}$), *ARP* (25.28 $\mu g_{DPPH}/\mu L_{extract}$) and *Y* (15.45 $g_{oil}/100g_{paste}$).

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