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INVESTIGACIÓN

Quantitative and qualitative effects of a pectolytic enzyme in olive oil production

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RESUMEN

Efectos cuantitativos y cualitativos de un enzima pectolítico en la producción del aceite de oliva.

Han sido efectuados ulteriores estudios sobre el empleo del «Olivex»®, una preparación enzimática pectolítica, en la extracción del aceite de oliva. Las pruebas tecnológicas han sido realizadas a nivel industrial con el sistema convencional de la presión, utilizando las variedades de aceitunas italianas «Leccino», «Dritta» y «Caroleo».

Los resultados de la investigación han confirmado que el «Olivex»® hace aumentar el rendimiento de la extracción, pero su efecto, con el antedicho sistema tecnológico, parecía menos evidente respecto a los sistemas continuos de centrifugación y de percolación-centrifugación, aplicados en pruebas anteriores. Además, el complejo enzimático ejercitaba una influencia positiva –aunque no muy significativa– sobre algunas características de calidad del aceite.

En efecto, el producto elaborado evidenciaba un contenido más alto de polifenoles, de o-difenoles, de *trans-2*-hexenales y de sustancias volátiles aromáticas. Además, los aceites mostraban mejores características organolépticas y evidenciaban valores más altos de la estabilidad oxidativa, del indicador global de calidad (I. G. C. 1), de la relación campesterol/stigmasterol, y del contenido de alcoholes triterpénicos. En cambio, los valores del indicador alcohólico y del contenido de hexanales, de alcoholes alifáticos y de hidrocarburos esteroideos resultaron más bajos.

Los valores apenas más altos del indicador de peróxido de los aceites representaban el único aspecto no positivo ligado al empleo de la formulación enzimática en el proceso de extracción.

PALABRAS-CLAVE: Aceite de oliva – Efecto cualitativo – Efecto cuantitativo – Enzima pectolítico – Extracción por presión.

SUMMARY

Quantitative and qualitative effects of a pectolytic enzyme in olive oil production.

The use of «Olivex»®, an enzymatic pectolytic preparation, was experimented in olive oil extraction, carried out at the industrial level with conventional pressed system. Tests were performed by processing «Leccino», «Dritta» and «Caroleo» varieties. The research results revealed that «Olivex»® increased the extraction yields, but its effect, with the above technological system, was less marked compared to the percolation and the centrifugation ones.

Furthermore such an enzymatic complex, exerted a positive influence –although not very significant– on the qualitative characteristics of the oils. In fact the product obtained pointed out a higher content of polyphenols, o-diphenols, *trans*-2-hexenal and aromatic volatile substances. It also achieved a higher sensory score and indicated higher values of the oxidative stability, the global quality index (G. Q. I. 1), the campesterol/stigmasterol ratio, and the triterpenic alcohol content. Instead the values of the alcohol index and the content of hexanal, aliphatic alcohols and steroid hydrocarbons, resulted lower.

The higher values of the peroxide index of the oils appeared as being the only negative aspect related to the use of the enzyme in the extraction process.

KEY-WORDS: Extraction by press – Olive oil – Pectolytic enzyme – Qualitative effect – Quantitative effect.

1. INTRODUCTION

As in other sectors of the food industry, in the field of the olive transformation different processing aids (enzymes, surface-active agents, absorbents, drainage materials and others) were experimented (Montedoro and Petruccioli, 1974; Leone *et al.*, 1977; Montedoro, 1987; Mendoza *et al.*, 1987; Federici *et al.*, 1988; Uceda, 1990; Servili *et al.*, 1992).

In Italy the first researches on the above extraction aids date back to the sixties. In spite of this they only rarely were used in olive processing. However, it is to be noted that their employment in Italy, at present day, is not legally recognized yet. The researchers mainly investigated the biological aids (enzymes), which at first were lyophilized preparations, which had to be rehydrated before their use. As well, to allow for their effect, the kneading operation had to be conducted at a temperature not lower than 30° C. But in the last ten years liquid enzymatic formulations were set up; they prevalently contain pectolytic enzymes and are more efficient; their use is simpler (because they do not have to be previously rehydrated and may be directly added to the olive paste).

«Olivex»® is one of the preparations that we have experimented. In previous reports (Ranalli and De Mattia, 1992; Ranalli and Costantini, 1994; Ranalli and Martinelli, 1994) some results, obtained by the above

enzymatic complex, were given. Formerly, we carried out the oil extraction trials by the percolation or centrifugation systems; in this paper the results achieved by the pressing system were related.

«Olivex»® is a pectolytic complex. It also contains cellulolytic and hemicellulolytic enzymes and exerts even other enzymatic activities. It is added to the olive paste in the crushing or kneading step; 20 ml of «Olivex»®, diluted with water (600 ml), per 0.1 tons of olives, are used. «Olivex»® degrades membranes of non crushed oily cells, permitting the recovery of the oil even from these. Even the vegetal colloidal structures are degraded. These structures bind the oil drops, wihch are thus liberated. A mass of free oil is then formed, which is easily extractable or recoverable by mechanical equipments. The oil, emulsified with the colloids, is contained, in the cytoplasm of the oleiferous cells; on the contrary, in the cell vacuole only free oil (~76%) is present. Furthermore «Olivex»® brakes oil-water emulsions, and induces positive effects on the rheological characteristics of the pastes. These latter are better centrifuged with a more efficient separation of the phases (oil, water and husk). *Aspergillus aculeatus* is the fungus through which «Olivex»® is obtained. This is a biological innocuous water-soluble product, and at the end of the extraction process, might thoroughly be present in the waste water.

2. EXPERIMENTAL

To carry out the experiment, three olive varieties («Leccino», «Dritta» and «Caroleo») were processed at the industrial level. An homogeneous sample of 1.8 tons of olives of good quality was processed, of which 0.9 tons by employing «Olivex»® and 0.9 tons without using the enzymatic complex (reference trials). Each half was divided into three equal parts, processed and tested.

Oil was extracted from de olives by using a pressing equipment and in general adopting the same operative conditions and the same processing diagram adopted in a previous experiment (Ranalli, 1989), the only important variation being that this time there was the use of the enzyme. The technological cycle based on only one pressing of paste was applied. In short the above operative conditions were:

– milling lenght 30'. To effect this operation a three stone-mill was used. The olive lots, before crushing, to removal of leaves and washing were subjected;

– kneading lenght 30'. During this step the oily paste was heated at 28°C;

– pressing lenght 90';

– maximum extracting pressure 380 kg/cms. To perform the pressing of the paste 16 inches hydraulic superpresses were used. The towers to press were made in such a manner that the radio among nylon filter diaphragms, paste layers and metal disks was of 4:3:1. To centrifuge the oily must an automated discarge centrifuge was used.

The enzyme was added to the olive paste during the milling step (the amount employed and the dilution ratio were those mentioned in the previous section).

During the testing, samples of olives, by-products and oil were taken. The olive processed has the following characteristics:

– «Leccino», oil 17.8 %, moisture 50.7 %, solids 31.5 %, number of olives per kg 682, weight of 100 olives g 163.1, average diameter of the drupes cm 1.16, pulpe/stone ratio 2.5, ripening index 3.6 (determined according to the method developped by the INRA of Jaen-Spain);

– «Dritta», oil 26.1 %, moisture 48.1 %, solids 25.8 %, number of olives per kg 579, weight of 100 olives 198.6, average diameter of the drupes cm 1.29, pulpe/stone ratio 4.6, ripening index 4.7;

«Caroleo», oil 25.0%, moisture 52.0%, solids 23.0%, number of olives per kg 260, weight of 100 olives 417.0, average diameter of the drupes cm 1.68, pulpe/stone ratio 5.3, ripening index 3.2.

The following analytical determinations were effected on the oils:

– analysis HRGC of the head space–volatile aromatic substances at 37°C. Internal standard 1-nonanol (Solinas *et al.,* 1987; Solinas *et al.*, 1988);

– analysis HPLC of the tocopherols (Angerosa, 1993);

– analysis HRGC of the phenolic fraction. Internal standard resorcinol (Solinas, 1987);

– analysis HRGC of the steroid hydrocarbons. Internal standard cholestadien (I.O.O.C., 1993a; I.O.O.C., 1993b);

– analysis HRGC of the waxes. Internal standard lauryl-arachidate (E.C., 1993);

– analysis HRGC of the fatty acids (E.C., 1991);

– analysis HRGC of the sterol fraction and the triterpene dialcohol. Internal standard cholestanol (E.C., 1991);

– analysis HRGC of the aliphatic and triterpene alcohol fraction. Internal standard arachidilic alcohol (E.C., 1991);

– analysis NMR of the triglycerides and the diglycerides (Sacchi *et al.,* 1990; Gunstone, 1991). The data obtained by this last analysis will be reported in another paper.

Furthermore, oil samples were pyrolised (Goodacre *et al.*, 1993) to achieve the mass spectra, which were statistically processed applying conventional methods of multivariate analysis (PCA, CVA, PCCV) and neural networks (ANN, KANN and others).

Other analytical observations, to which the oils were submitted concern; the free acidity, the peroxide index, the Watts and Major's carbonyl index, the total polyphenols, the o-diphenols (by colorimetry), the limpidity, the U. V. spectrophotometric indices, the ΔK, the Wolff's ratio (R), the chlorophylls *a* and *b*, the

phaeophytins *a* and *b*, the chlorophyllic colour index, and the carotenoid colour index.

To determine the colour of the oils, the C.I.E. method by using the tri-chromatic coordinates, was applied to assess, through transmittance measurements, in addition to the above chromatic indices, the brightness or value (h %), the purity or chroma (s%), the hue (ld). The Naudet's integral colour index and the colour ratio of the oils was also calculated.

The product was then submitted to sensory tasting by adopting the panel method and relative scores were statistically compared (difference test). The panelists, in addition to the sensory attributes, also considered the nuances as well as the typical characteristics and any off-flavours (if present). In addition, data was acquired on the shelf-life of the oils by evaluating their oxidative stability, i. e. the induction time of the peroxidation reactions (by means of «Rancimat» instrument, which automatically applies the accelerated Swift's test).

Finally, the total quality of the oils was evaluated, by calculating: (i) the global quality index (G.Q.I. 1), by using the linear equation proposed by the I.O.O.C. (1990), based on four canonical variables, one of which is the sensory score to which the highest ponderal coefficient is attributed, resulting in the variable having the largest relative weight on the overall oil quality; (ii) the global quality index (G.Q.I. 2), by using the algorithm developped by Solinas *et al.*, (1992), in which a fifth variable is included, i.e. the total polyphenols, which should otherwise be considered redundant as it is correlated with the sensory score.

Some parameters and indices were determined by the analytical methods indicated by the E.C. regulations N.° 2568/91 (E.C., 1991) and relative amendments. The other chemical and physical analysis were performed by following the methodologies pointed out in other reports (Ranalli, 1991; Ranalli, 1992).

3. RESULTS AND DISCUSSION

The results obtained are given in Tables I, II, III, IV, V, VI.

3.1. Technological quantitative results

«Olivex»®, with «Leccino» and «Dritta» varieties, on average led to obtain higher yields, while with «Coratina» variety no difference was observed in

Table I

Oil outputs and analytical characteristics of the by-products obtained by processing with the pressing system three olive varieties by using the enzymatic complex «Olivex»®. Comparison with the results of the reference trials

Technological parameters	«Leccino» variety		«Dritta» variety		«Caroleo» variety	
	Processed with «Olivex»®	Reference trial	Processed with «Olivex»®	Reference trial	Processed with «Olivex»®	Reference trial
Olives						
Oil extraction outputs (olive weight base) (Kg/ton)	152	146	226	222	222	222
Oil extraction yields (fruit oil base) (%)	85.4	82.0	86.6	85.1	88.8	88.8
Husk						
Moisture (g/Kg)	237	258	250	254	242	257
Residual oil (g/Kg)	58.2	69.0	78.8	80.3	72.7	88.4
Residual oil (g/Kg DM)	76.3	93.0	105.1	107.7	95.9	119.0
Pulp/stone ratio (% DM)	79.9	62.3	202.1	140.4	271.7	171.0
Effluent						
Dry matter (g/l)	142	146	128	144	115	118
Oil (g/l)	1.26	4.37	1.86	2.67	1.38	2.43
Oil (g/Kg DM)	8.8	29.9	14.5	18.4	12.0	21.2
Phenols (g/l, caffeic acid)	9.9	9.6	11.4	11.0	8.0	7.6
O-diphenols (g/l, caffeic acid)	4.1	3.9	3.4	3.1	2.7	2.5
Turbidity (NTU)	2015	5145	9343	13949	3239	6225
COD (g O_2/l)	203.1	224.6	196.9	209.2	144.6	156.9

DM= Dry matter

NTU= nephelometer turbidity unit

comparison to the reference test. With the pressing extraction system, «Olivex»® achieved less significant results in comparison to the percolation or centrifugation system (Ranalli and De Mattia, 1992; Ranalli and Costantini, 1994; Ranalli and Martinelli, 1994). The experiments by adopting these last systems were formerly effected.

In fact, with the pressing system, for olive grinding, the mill-stone is used (rather than mechanical crushers), and no water is added to the paste, consequently the formation of the emulsion is minor. For this reason the effect of the enzyme on the emulsion is less significant.

The higher outputs obtained by the enzyme, by the lower oil amounts found in the by-products, is confirmed. Then, with «Olivex»® a less humid husk was produced, but not with a very important difference, because the pressed husks are poorer in moisture in comparison to those from continuous systems. The husk, when «Olivex»® was employed, was also characterized by a higher pulp/stone ratio. Finally, because of the enzyme, the dry residue, turbidity and COD values ascertained in the effluent, resulted lower, while the phenol and o-diphenol content appeared higher (the oils, as will later be pointed out, also appeared richer in natural antioxidants). The positive effects of «Olivex»® on the quantitative results, appeared supported even by the other analytical parameters, above cited, determined on the by-products.

3.2. Technological qualitative results

3.2.1. *Acidity, peroxide index, UV spectrophotometric indices, Watts Majors's index, turbidity*

These analytical parameters and indices determined on the oils, in general, were not very influenced by the enzyme, with the exception of the peroxide index, which resulted as being higher when the olive pastes were treated by «Olivex»®. This phenomenon, even with the centrifugation system appeared evident (Ranalli and Costantini, 1994; Ranalli and Martinelli, 1994). The oil turbidity, however, was also significantly affected because its values was lower.

3.2.2. *Total poliphenols, o-diphenols, tyrosol, hydroxytyrosol, tocopherols*

The pectolytic enzyme led to achieve oils with a total content of polyphenols and o-diphenols constantly higher. With «Leccino», «Dritta» and «Caroleo» varieties, the phenol concentration of the oils increased by about 13%, 7% and 20% respectively. This is because the complex molecular structures of the parenchymatic tissues of the drupes are probably degraded by the enzyme. Thus the liberization of the phenolic constituents (in the above tissues plentifully

Table II
Analytical characteristics of the oils obtained by processing with the pressing system three olive varieties by using the enzymatic complex «Olivex»®. Comparison with the reference oils

	«Leccino» variety		«Dritta» variety		«Caroleo» variety	
Analytical parameters determined in the oils	**Oil extracted with «Olivex»®**	**Reference oil**	**Oil extracted with «Olivex»®**	**Reference oil**	**Oil extracted with «Olivex»®**	**Reference oil**
Acidity (g/Kg, oleic acid)	4.4	5.3	4.7	5.2	3.4	2.8
Peroxide index (meq O_2/Kg)	8.6	6.9	11.4	8.2	10.0	8.5
Carbonyl index	6.82	7.11	3.96	3.94	3.39	2.99
Total poliphenols (mg/l, caffeic acid)	277	241	281	262	221	176
O-diphenols (mg/l, caffeic acid)	195	169	170	168	150	126
«Rancimat» stability (h)	18.5	17.0	12.8	12.0	16.4	14.4
Turbidity (NTU)	26	72	19	20	1330	1860
K232	1.552	1.810	1.513	1.522	1.696	1.415
K270	0.145	0.134	0.146	0.151	0.098	0.073
$\Delta Kx10^3$	-6	-6	-5	-4	-4	-3
R	10.7	13.5	10.4	10.1	17.3	19.3
Panel test (score)	8.1	7.5	8.2	7.7	7.9	7.3
Global quality index (GQI1)	8.0	7.6	7.9	7.5	8.2	7.9
Global quality index (GQI2)	37.1	36.8	36.2	36.3	37.1	37.2
Chlorophylls and phaeophytins (mg/Kg)	11.0	11.3	7.4	8.2	6.9	6.7
Chlorophyllic colour index (%)	46.7	48.4	30.6	34.5	28.8	28.3
Carotenoid colour index (%)	126.9	121.5	77.6	87.3	86.1	74.5

present), and their solubilization in the oil (and in the waste water, see previous section), is major. Instead the tocopherol content, as well as the individual phenol content (tyrosol and hydroxytyrosol) of the oils, did not result very influenced by the enzyme complex. In fact, its effect on these components appeared either positive or negative, in function of the olive variety. Oils richest in polyphenols were obtained from «Dritta», while the product richest in tocopherols were achieved from «Leccino».

3.2.3. *Panel test, «Rancimat» stability, global quality indices (G.Q.I. 1 and G.Q.I. 2)*

With all three olive varieties processed, the sensory score obtained for the oils, appeared higher when the olive pastes were treated by the enzymatic complex. Even the oxidation resistance resulted major, from which a positive incidence of the enzyme on the shelf-life of the product might be hypothesized. In fact, as is well known, these parameters are correlated with the phenolic concentration (noted above). With «Leccino», «Dritta» and «Caroleo» varieties, the induction time of the peroxidation reactions increased by about 8.8%, 6.7% and 13.9%, respectively. Similarly the global quality indices values appeared higher in the oil produced by the enzyme formulation. Oils originating from «Dritta», «Leccino» and «Caroleo» varieties were characterized respectively by: highest sensory score, highest growing rancid resistance, highest global quality indices values.

3.2.4. *Aromatic volatile fraction*

Oils obtained by the enzyme pointed out a higher average content of aromatic volatile substances. An analogous phenomenon was observed for the *trans*-2-hexenal. This is the main component of the volatile fraction and, together with the phenolic compounds, is the principal factor which determines the «fruttato» of the oils. On the other hand «Olivex»®, led to obtain oils with a lower content of hexanal (substance having an unpleasant aroma). In regards to the other volatile substances, on the whole N.° 21(those identified), see Table III and Figure1. Oils with the highest content of aromatic compounds, were achieved by «Leccino» variety. A less aromatic product was obtained by «Dritta» and «Caroleo» varieties.

Table III

Content of aromatic volatile substances (expressed in mg/Kg) identified in the oils obtained by processing with the pressing system three olive varieties by using the enzymatic complex «Olivex»®. Comparison with the reference oils

	«Leccino» variety		«Dritta» variety		«Caroleo» variety	
Aromatic volatile substances identified in the oils	**Oil extracted with «Olivex»®**	**Reference oil**	**Oil extracted with «Olivex»®**	**Reference oil**	**Oil extracted with «Olivex»®**	**Reference oil**
n-Octane	6.6	10.3	2.4	29.0	18.1	39.8
Etyl-acetate	1.1	0.6	0.3	2.4	3.2	3.6
2-Methyl-butyraldeyde	3.6	5.7	0.1	2.3	ND	3.1
3-Methyl-butyraldeyde	5.0	7.9	0.1	2.4	ND	1.5
Ethanol	17.7	16.5	2.4	33.9	79.5	50.2
3-Pentanone	19.3	6.1	3.3	32.2	30.2	31.1
1-Penten-3-one	11.1	8.3	0.7	6.1	3.9	5.3
Hexanal	22.2	28.9	4.6	38.0	14.8	26.8
Isobutil alcohol	1.7	2.3	0.8	7.2	12.4	10.5
Trans-2-pentenal	1.5	1.3	0.3	1.1	ND	ND
1-Penten-3-ol	9.4	7.3	1.3	12.5	12.1	12.7
Iso amyl alcohol	5.8	6.6	2.3	18.4	42.1	29.7
Trans-2-hexenal	534.0	438.5	534.0	255.1	43.9	121.1
n-Amyl alcohol	1.2	0.9	0.1	1.3	1.8	1.8
2-Penten-1-ol	7.6	6.1	1.0	10.4	8.3	9.2
1-Hexanol	15.0	10.0	4.0	32.3	34.4	40.8
3-Exen-1-ol (*cis*?)	7.4	4.7	0.8	8.1	107.7	77.5
Trans-2-hexenol	30.1	26.6	4.0	48.0	28.5	45.0
Acetic acid	3.1	1.9	0.3	1.5	1.8	2.4
1-Octanol	3.6	3.6	1.8	4.8	22.4	5.6
2-Butanol	33.3	3.5	1.7	2.6	24.7	2.4
Total volatile substances	773.1	634.3	684.5	599.1	525.3	555.7

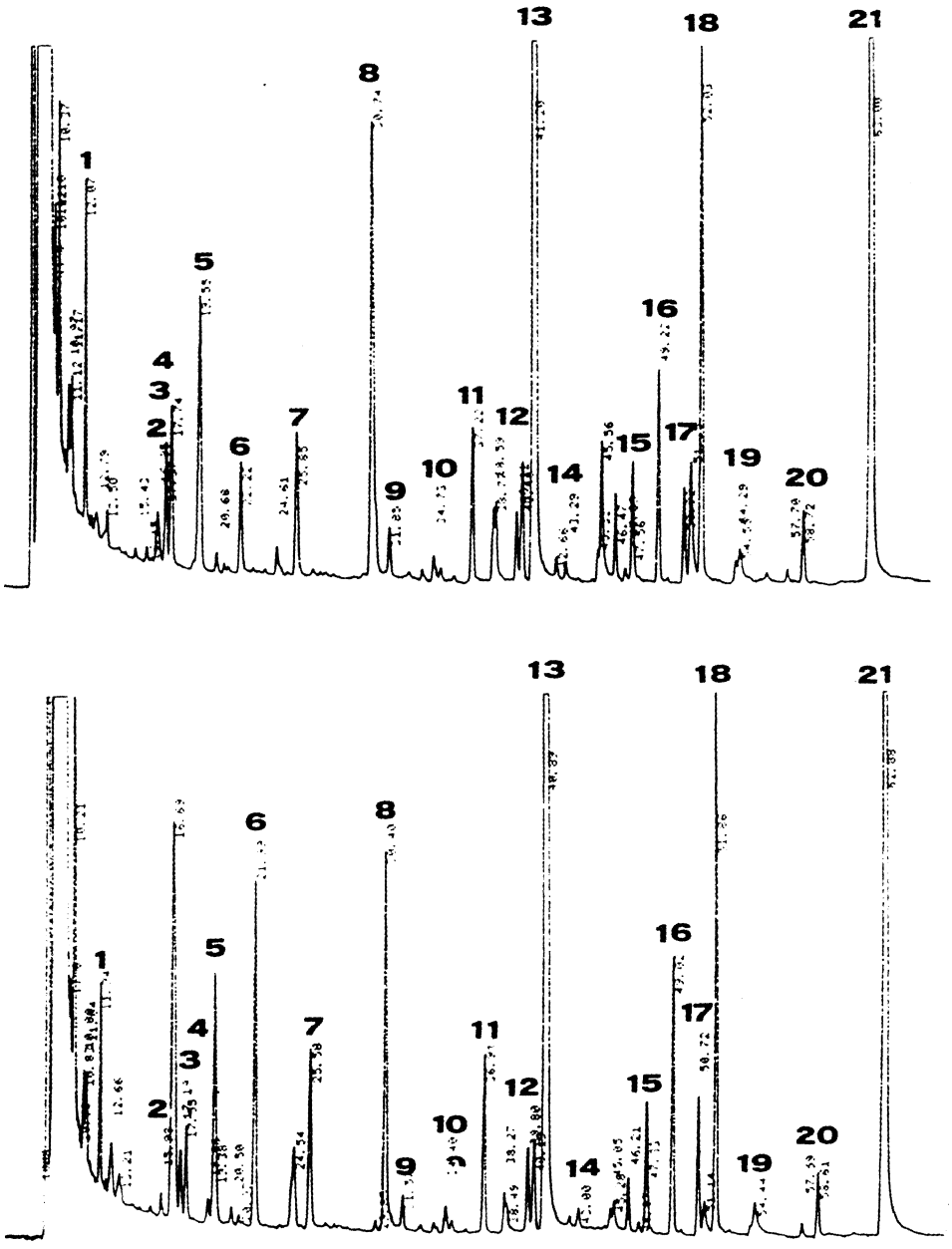

Figure 1
Aromagrams of two oils, both obtain from «Leccino» variety. Above the reference oil. Below the oil produced with «Olivex» (R). Peaks identified: 1) n-Octane, 2) Etyl-acetate, 3) 2-Methyl-butyraldeyde, 4) 3-Methyl-butyraldeyde, 5) Ethanol, 6) 3-Pentanone, 7) 1-Penten-3-one.8) Hexanal, 9) Isobutil alcohol, 10) Trans-2-pentenal, 11) 1-Penten-3-ol, 12) Iso amyl alcohol, 13) Trans-2-hexenal, 14) n-Amyl alcohol, 15) 2-Penten-1-ol, 16) 1-Hexanol, 17) 3-Exen-1-ol (cis?), 18) Trans-2-hexenol, 19) Acetic acid, 20) 1-Octanol, 21) 1-Nonanol (internal standard)

3.2.5. *Chromatic characteristics, lipochrome content of the oils, spectrophotometric curves in the visible zone between 400-700 nm*

The chromatic parameters and indices values of the oils, produced by the enzyme, resulted either slightly higher or slightly lower, as they were only dependent on the olive variety processed. The values of ld(hue), did not completely differ from the reference oil, indicating that both oil types were characterized by the yellow colour, which clearly prevailed with respect to the green. This was confirmed by the values of the colour ratio (between the chlorophill absorbance and carotenoid absorbance), which, with the olive varieties considered, fluctuated from 2.8 to 3.1. «Leccino» variety gave oils most coloured in comparison to the «Dritta» and «Caroleo» varieties.

3.2.6. *Steroid hydrocarbons and waxes*

The analytical observations conduced pointed out that:

– the content of the steroid hydrocarbons was frequently lower in the oils obtained from the olive paste treated by the enzyme;

– in any oil produced non-detectable quantities of campestadien were present;

– the stigmastadien content of the oils usually exceeded the limit value (0.10 mg/kg);

– «Leccino» variety gave oils with the highest hydrocarbons and waxe content;

– the average content of individual or total waxes, apart from the olive variety, in the two oil types, resulted as being similar, but tendentially higher in the reference oil.

It is to be noted that the above constituents, hydrocarbons and waxes, are correlated with the genuiness of the oils.

Table IV

Other analytical characteristics of the oils obtained by processing with the pressing system three olive varieties by using the enzymatic complex «Olivex»®. Comparison with the reference oils

	«Leccino» variety		«Dritta» variety		«Caroleo» variety	
Analytical parameters determined in the oils	**Oil extracted with «Olivex»®**	**Reference oil**	**Oil extracted with «Olivex»®**	**Reference oil**	**Oil extracted with «Olivex»®**	**Reference oil**
Brightness (%)	67.3	66.8	74.9	74.5	78.3	79.7
Chroma (%)	87.8	86.7	71.6	76.1	74.7	69.4
Hue (nm)	578	577	577	577	577	576
Colour ratio (abs 446/abs 668)	2.9	2.8	2.8	2.8	3.1	2.8
Integral colour index	15.1	15.2	9.0	9.7	7.9	6.8
Tyrosol (mg/Kg)	11.9	8.4	10.5	4.8	8.5	12.9
Hydroxytyrosol (mg/Kg)	5.1	3.5	6.4	4.7	10.2	11.3
Tocopherols (mg/Kg)	216.5	203.9	73.9	93.1	87.9	89.9
a-Tocopherol (mg/Kg)	216.2	203.5	73.7	92.9	87.6	89.8
g-Tocopherols (mg/Kg)	0.3	0.3	0.2	0.2	0.2	0.1
Steroid hydrocarbons (mg/Kg)	0.71	5.00	0.36	0.58	0.43	ND
Campestadien (mg/Kg)	ND	0.19	ND	0.12	ND	ND
Stigmastarien (mg/Kg)	0.12	4.44	0.09	0.17	0.13	ND
Stigmastadien (mg/Kg)	0.59	0.37	0.27	0.29	0.30	ND
Waxes (mg/Kg)	169	168	98	113	111	104
C40 (mg/Kg)	54	57	34	35	34	34
C42 (mg/Kg)	46	50	22	30	28	28
C44 (mg/Kg)	24	24	15	17	15	14
C46 (mg/Kg)	45	37	27	31	34	28

3.2.7. *Composition of the sterol fraction, campesterol/stigmasterol ratio, triterpene dialcohols*

The analitical data relating to these components showed that:

– the campesterol/stigmasterol ratio (considered an index of quality), in the oils obtained by the enzyme, was constantly higher;

– the cholesterol percentage of the oils, globally considered, only in one case exceeded the limit value (0.5%);

– similarly the Δ^7-stigmastenol percentage of the oils only in one case exceeded the limit value (0.5%);

– the total sterol content, in the oils produced from the «Dritta» variety, did not exceed the 1g/kg value, which is the minimun limit set by the E.C. norm;

– the composition of the sterol fraction was significantly influenced by the olive varieties processed;

– the oils originating from «Dritta» variety indicated the highest content of triterpene dialcohols.

3.2.8. *Aliphatic and triterpene alcohol fraction. Alcohol index*

In regards to the composition of the alcohol fraction of the oils, when the enzyme was employed, it was observed that:

– the values of the alcohol index (which too is a quality index and with which it is inversely correlated) regularly resulted lower;

– the aliphatic alcohol content was frequently lower;

– the triterpenic alcohol content frequently appeared higher.

Besides, the analytical data indicated that, on the composition of the alcohol fraction of the oils, a significant influence exerted the olive variety processed.

3.2.9. *Fatty acids composition*

The use of «Olivex»®, in the extraction process, had no incidence on the composition of fatty acids of the oils, which instead was influenced by the olive variety.

Table V

Sterol and triterpene dialcohol fraction composition (%) of the oils obtained by processing with the pressing system three olive varieties by using the enzymatic complex «Olivex»®. Comparison with the reference oils

Sterol and triterpenic dialcohol components of the oils	«Leccino» variety		«Dritta» variety		«Caroleo» variety	
	Oil extracted with «Olivex»®	Reference oil	Oil extracted with «Olivex»®	Reference oil	Oil extracted with «Olivex»®	Reference oil
Cholesterol	0.2	0.3	0.3	0.7	0.1	0.3
Brassicasterol	0.1	ND	0.2	0.1	0.2	0.3
24-Methylenecholesterol	0.1	ND	0.2	0.1	0.2	0.3
Campesterol	2.8	3.0	2.9	2.8	1.6	1.7
Campestanol	0.3	0.3	0.5	0.5	0.4	0.4
Stigmasterol	0.7	0.8	1.0	1.0	0.4	0.5
Δ^7-Campesterol	0.5	ND	0.2	ND	0.1	0.4
Δ^5-23-Stigmastadienol	ND	ND	ND	ND	ND	ND
Chlerosterol	1.1	1.0	1.1	0.8	1.2	1.6
b-Sitosterol	82.7	82.6	74.8	80.7	71.5	73.2
Sitostanol	1.4	1.4	1.4	2.3	0.8	0.9
Δ^5-Avenasterol	8.5	9.1	15.5	8.1	22.9	20.0
Δ^5-24-Stigmastadienol	0.9	0.3	0.8	2.2	0.2	0.2
Δ^7-Stigmastenol	0.3	0.8	0.4	ND	0.2	0.2
Δ^7-Avenasterol	0.3	0.8	0.4	ND	0.2	0.2
Triterpene dialcohols	0.9	1.0	3.3	3.3	1.8	1.5
Total b-sitosterol	94.6	94.4	93.6	94.1	96.6	95.9
Total sterols (mg/Kg)	1553	1626	988	744	1287	1347
Campesterol/stigmasterol ratio	3.9	3.8	3.0	2.7	3.6	3.1

Table VI
Fatty acids composition (%) and aliphatic and triterpenic alcohol content (mg/Kg) of the oils obtained by processing with the pressing system three olive varieties by using the enzymatic complex «Olivex»®. Comparison with the reference oils

Fatty acids and alcoholic components of the oils	«Leccino» variety		«Dritta» variety		«Caroleo» variety	
	Oil extracted with «Olivex»®	Reference oil	Oil extracted with «Olivex»®	Reference oil	Oil extracted with «Olivex»®	Reference oil
C14: 0	ND	ND	ND	ND	ND	ND
C16: 0	13.0	12.7	13.4	13.4	14.0	13.3
C16: 1	1.1	1.1	1.1	1.2	1.7	1.7
C17: 0	ND	ND	ND	ND	0.1	0.1
C17: 1	0.1	0.1	0.3	0.1	0.3	0.3
C18: 0	2.1	2.3	3.4	3.2	2.8	2.7
C18: 1	75.2	74.3	69.9	70.4	72.9	73.7
C18: 2	7.1	8.2	10.4	10.2	6.8	7.0
C18: 3	0.7	0.7	0.6	0.6	0.4	0.4
C20: 0	0.3	0.2	0.5	0.5	0.4	0.4
C20: 1	0.3	0.3	0.3	0.3	0.4	0.3
C22: 0	0.1	0.1	0.1	0.1	0.2	0.1
C24: 0	ND	ND	ND	ND	ND	ND
Saturated acids/unsaturated acids ratio	0.18	0.18	0.21	0.21	0.21	0.20
Oleic acid/linoleic acid ratio	10.6	9.1	6.7	7.0	10.6	10.5
Alcoholic index	0.11	0.36	0.07	0.21	0.02	0.03
Aliphatic alcohols	92	142	71	88	40	37
C22	22	34	11	13	8	7
C24	40	56	19	26	13	12
C26	22	34	29	34	13	12
C28	8	18	12	15	6	6
Triterpenic alcohols	758	827	1011	916	748	598
b-amyrin + butirrospermol	92	94	133	88	59	67
Cycloartenol	280	293	250	220	177	127
24-Methylenecycloartanol	386	440	608	628	404	512

Finally, it is important to point out that the multivariate statistical analysis of the data and the mass spectra (obtained by pyrolising the oil samples), carried out, applying both conventional and neural methods, showed how the oil samples were differentiated by olive variety. Furthermore, the oils of the same variety, were differentiated in regards to the modality with which they were obtained, indicating that the enzyme led to achieve oils qualitatively different from those produced by the reference process.

4. CONCLUSIONS

The employment of «Olivex»® –a pectolytic preparation– in olive processing, carried out by the pressing system, led to achieve a increase of the yieds, which however appeared less significant in comparison to the percolation and centrifugation systems (the trials by applying these last systems were effected formerly). Furthermore the enzymatic complex an iprovement (although not very significant) of the oil characteristics induced.

In fact the product was characterized by the:

- –lower values of turbidity;
- –higher content of poliphenols and o-diphenols;
- –higher sensory quality;
- –higher oxidation resistance (permitting to hypothesized for it a longer shelf-life);
- –higher values of global quality index (GQI1);
- –higher content of *trans*-2-hexenal and total volatile aromatic substances;
- –lower content of 2-hexanal;
- –lower content of total steroid hydrocarbons;
- –higher values of campesterol/stigmasterol ratio;
- –lower values of alcohol index;
- –lower content of aliphatic alcohols;

–higher content of triterpene alcohols;

–higher values of peroxide index (this was the only non-positive aspect connected with the employment of the enzyme in the extraction process).

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