

Characterization of extra virgin olive oils obtained from different cultivars

Opis svojstava ekstra djevičanskog maslinovog ulja dobivenog iz raznih kultivara

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ABSTRACT

The determination of some minor components of extra virgin olive oil, and in particular of the polyphenolic fraction of pigments and fragrances, can contribute to the characterization of the monovarietal productions: the quali-quantitative assessment of these substances can in fact contribute to the valorisation of typicalities and, at the same time, consent to the optimisation of the operational techniques during the processing and the eventual blending.

The analytical techniques, applied here for the characterisation of extra virgin olive oil of Abruzzo and Istria, have highlighted that chemical composition can enhance the valorisation of these typical products. Nevertheless, further sampling program is needed to evidence typical composition profile that might be used as "origin markers".

Key words: extra virgin olive oil, cultivar

SAŽETAK

Određivanje nekih manjih komponenata ekstra djevičanskog maslinovog ulja, naročito polifenolne frakcije pigmenta i mirisa, može doprinijeti svojstvima monovarijetetnih proizvoda: kvalitativna i kvalitativna procjena tih supstancija može zapravo doprinijeti valorizaciji tipičnosti te, istodobno omogućiti optimizaciju operativnih tehnika za vrijeme prerade i miješanja.

Analitičke tehnike, ovdje primijenjene za opis svojstava ekstra djevičanskog maslinovog ulja iz Abruzzo i Istre pokazuju da kemijski sastav može popraviti vrijednost ovih tipičnih proizvoda. Ipak, potreban je dodatni program uzorkovanja da se dokaže tipični profil sastava, što se može upotrijebiti kao "oznaka podrijetla".

Ključne riječi: ekstra djevičansko maslinovo ulje, kultivar

INTRODUCTION

The product characterization of the oils obtained from different cultivars is fundamental to obtain high quality production and for the valorisation of typicalities.

It is known that the quality of virgin olive oils, above all concerning product, technological and nutritional aspects, is a function of the characteristics of the composition and, more specifically, of the concentration of anti-oxidants.

In this paper, we describe the results of the analysis of “minor components”— polyphenols, pigments and fragrances, valuable parameters of the quality of the oil, not yet officially recognised but certainly carry considerable importance in extra virgin olive oils obtained from the cultivar typical of Abruzzo (Italy) and Istria (Croatia).

In the last ten years the study of the phenolic compounds of olive oil has gained a remarkable applicative interest for the effects that these substances have on the quality, the stability of the product and for the intrinsic nutritional qualities. Numerous studies have been conducted on this minor polar fraction with the double aim of isolating and identifying the single components and of defining the antioxidant and biological activity as well as the organoleptic properties. Both in the leaves and in the olives flavonoid glucosides are present, phenolic glucosides, derivatives of cinnamic and caffeic acid which are part of the minor components fraction.

The research conducted on olive oil chemical composition highlights that the polyphenols are subject to remarkable variations according to the variety, to the agronomic conditions, the state of ripeness and to the technology of conservation. In the oil, residuals in more limited quantities are found in the form of simple phenolics and aglycons, in quantities of 50-500 mg/hg (Montedoro et al., 1992). The presence of natural pigments (chlorophylls, pheophytins, carotene) is relevant both with respect to the product and technological characteristics and with respect to the stability of the product. Moreover a green colour is often appreciated by consumers.

It is known, in fact, that these substances can have a remarkable influence on the preservability of the product as pro-oxidants in synergy with possibly present metals (Capella P. et al 1991). The concentration of these pigments is a function of several variables, both agronomic and technological.

Under the influence of UV radiation chlorophylls and pheophytins act as catalysts in the formation of singlet oxygen (Kiritsakis A. et al., 1985) and

therefore favour the first phases of the process of self-oxydation. Some researches, however, highlight the delaying role played by carotenes with respect to photo-oxidation (Kiritsakis A. et al., 1995).

Traditionally the tenor of these compounds is determined with spectrophotometric methods by measuring total chlorophylls and total carotene oils with values in the range from 1-10 ppm, regarding the chlorophylls, and from few up to 100 ppm for the carotene oils (Vitagliano M., 1982) (Wolff J. P., 1968); in fact the profile of the absorbance curve of the virgin olive oils in the visible range is well known and characterised by typical bands for the chlorophylls and the carotenoids.

Meaningful results have been obtained with the HPLC analysis (M. I. Minguez-Mosquera, 1992).

Vast literature exists on the volatile compounds of olive oils. Many of the papers written on this topic have the purpose of investigating the qualitative composition of the aromatic components both of the olives and of the oil extracted with diverse available technologies, also in relation to the agronomical, varietal and geographic variables which results in conditioning the product characteristics of the final product.

Several researches have been conducted on those volatile components that originate from oxidative processes and that assume a relevant meaning in the sensorial profile of the product, in as much as they are responsible for unpleasant sensations defined as “faults” from the panel test (regulation CEE 2568/91 and successive modifications and integrations). Furthermore, recent methods DHS-GC-MS linked to panel tests have been proposed (E. Psomiadou, M. Tsimidou, 2001) (B. Gandul et al., 1996), (M.I. Minguez et al., 1990), (M.I. Minguez et al., 1989), (Procida et al. 2004).

MATERIALS AND METHODS

Oil Samples. EVOO samples were obtained from fruits of several varieties cultivated in Abruzzo and Croatia (Table 1). Olive fruits were harvested in the years 2001 and 2002 at the medium ripening stage, and the relative oils were immediately obtained by crushing the olives by continuous processing techniques. All the analyses were carried out in the period September-December 2002.

SPE extraction of the Phenolic Fraction. Commercially available octadecyl C₁₈ cartridges (1 g. 6 ml) (International Sorbent Technology, UK)

were used for the extraction of the phenolic fraction according to the following protocol: 1 g. of olive oil was dissolved in 10 mL. of hexane, and put onto a column previously conditioned with 2 x 10 ml of methanol and 2 x 10 ml of hexane. The column was eluted with 4 x 10 ml of hexane to eliminate all the lipophilic fractions, and the retained polar compounds were recovered by eluting with 4 x 10 ml of methanol. Trapping and release of analytes from C₁₈ solid phase was demonstrated to be highly competitive with liquid/ liquid extraction procedure (Liberatore *et al.*, 2001).

Table 1. Description of the samples, list of abbreviations, year of sample collection and extraction system used.

Tablica 1. Opis uzoraka, popis kratica, godina uzimanja uzoraka i korišten sustav ekstrakcije

Year 2001			
Identifying abbreviation	Cultivar	Place of origin	Extraction system
322	DRITTA	Loreto Aprutino (PE)	Continuous
403	LECCINO	Rocca San Giovanni (CH)	Continuous
406	GENTILE	Rocca San Giovanni (CH)	Continuous
416	PICHOLINE	Veglia (Croatia)	Continuous
419	BIANCHERA	Pula (Croatia)	Continuous
Year 2002			
Identifying abbreviation	Cultivar	Place of origin	Extraction system
32	DRITTA	Loreto Aprutino (PE)	Continuous
41	GENTILE	Rocca San Giovanni (CH)	Continuous
44	LECCINO	Rocca San Giovanni (CH)	Continuous
91	BIANCHERA	Pula (Croatia)	Continuous
92	PICHOLINE	Porec (Croatia)	Continuous

Total Polyphenols Determination. The total polyphenol content of the methanol extracts was evaluated colorimetrically using the Folin-Ciocalteu reagent. The method was adapted from Singleton and Rossi (Singleton *et al.*, 1965). A diluted extract (0.5 ml of 1:10. v/v) or phenolic standard was mixed with Folin- Ciocalteu reagent (5 ml. 1:10 diluted with Nanopure water) and aqueous Na₂CO₃ (4 ml. 1 M). Solutions were maintained at room temperature for 60 min. and the total polyphenols were determined colorimetrically at 725 nm. Gallic acid standard solutions were used to calibrate the method.

Extraction process of phenolic extracts (lignans). According to Pirisi *et al.* (2000), exactly 2 g. of oil were weighed in a *Sovirel* screw cap test tube, 1 ml of *n*-hexane, 2 ml of methanol/water mixture (60/40, v/v) were added and, after an agitation in *Vortex* for 1 minute, the sample was subjected to a centrifugation for 5 minutes at three 3000 (radius 15 cm).

Afterwards, the hydro alcoholic phase which had separated, was taken and the lipidic fraction, still in *n*-hexane, subjected to two further washings with the same mixture. In order to remove any possible oil residuals, the three reassembled hydro alcoholic phases were subjected to a final washing with 1mL of *n*-hexane and, after a further centrifugation for 5 minutes at 3000 revolutions, the extracted phenolic fraction was vacuum-packed at 35°C with *Rotavapor*.

The phenolic fraction was then taken again in 0.5 ml of methanol/water (50/50, v/v), filtered using porous nylon filters of 0.45 µm and stored in a freezer at -18° C.

HPLC analysis of lignans. The two main components of lignans – (+) – pinoresinol and (+) – 1 – acetoxypinoresinol – were determined in liquid chromatography combined with detectors DAD and MS using UV spectrum and mass spectrum respectively for their identification.

The identity has been established through the comparison of the mass spectrum and the fragmentations obtained with the identification reported by *Owen et al.* (2000).

Furthermore, the identity of lignans was confirmed by comparing the UV spectrums to the UV spectrums reported by *Brenes et al.* (2002).

Pigments extraction. The analytic method defined by M.I. Minguez Mosquera was used. It considers a preventive separation (division liquid-liquid) of the pigments to be examined with hexane and N,N-dimethylformamide; lipids and carotenes are included in the hexanic fraction and chlorophylls, chlorophyllic derivatives like pheophytins and xanthophylls, in N,N-dimethylformamide. This fraction is then treated with a solution of Na₂SO₄ at 2% and extracted again with a mixture 1:1 made by hexane and diethyl ether. Of the two obtained phases, one is organic and the other one is aqueous. The aqueous phase is discarded by eliminating polyphenols and other water-soluble compounds. The organic phase is dry-cleaned and suspended once more with acetone for the injection in HPLC.

Analysis of chlorophylls in HPLC. The separation was obtained by using a Waters column, the Spherisorb ODS-2. As a detector, a fluorescent spectrometer (Perkin-Elmer, excitation wavelength and emission respectively

440 and 660 nm) for chlorophylls and pheophytins was used. The xantofills were measured instead by using a UV/Visible (LC95, Perkin Elmer, wavelength used 430 nm) detector.

Volatile compounds extraction. The head space sampling technique used is the one assessed by Barcarolo *et al.* (1992). Each sample (7 ml) was weighted into a 10 ml vial to which internal standard (isooctane 13.8 µg) was added, then the vials were sealed with aluminum-rubber septum. Vials with oil were conditioned at 40°C for 15 min before analysis, then stripping was carried out for 150 s. Stripping was realized with helium, at a rate of 10 ml/min. Volatile components were driven into a capillary tube inside a cryogenic trap (liquid nitrogen) that could be cooled at -110°C, in a column mode to a capillary gas chromatograph Fisons GC 8000.

GC-MS analysis. At the end of sampling time, desorption of volatile components takes place, by heating of the trap to 240°C and transfer of analytes to the analytical column. The analytical column used is a capillary fused-silica column 50 m X 0.32 mm I.D., coated with PS 264, 3µm film thickness. The capillary GC system is coupled directly to a MD 800 mass spectrometer (Fisons). GC conditions were the following: oven initial temperature 40°C, held for 6 min, then programmed to 180°C at a rate of 5°C/min, then 5 min at 180°C. Transfer line temperature was kept at 200°C.

A mass spectrometer scanned from m/z 29 to m/z 300 at 0.5 s cycle time. The ion source was set at 180°C and spectra was obtained by electron impact (70 eV).

RESULTS AND DISCUSSION

Polyphenols

Table 2 reports on the figures of the total polyphenols, lignans, pinosresinol and acetoxyl-pinosresinol, as well as their relation.

The phenolic fraction analysis of monovarietal oil produced with olives of *cvv.* Dritta, Leccino, Gentile, Bianchera and Picholine, harvested in a medium stage of growing, highlighted the presence of components of phenolic substances belonging to the category of simple phenols, like hydroxy tyrosol, tyrosol and deacetox oleuropein and of two phenolic substances belonging to the family of lignans as the (+)-pinosresinol and the (+)-1-acetoxypinosresinol.

Table 2. Polyphenols and Lignans in the examined oil

Tablica 2. Polifenoli i lignini u istraživanom ulju

Years	Samples	MEDIUM like mg di 3,4-DHPAA / kg oil			Relation 1 A/P	Poliphenols total	Cultivar
		Pinoresinol	1-Acetoxy Pinoresinol	Total			
2001	322	2,7	27,6	30,3	10,6	390	Dritta
	403	3,6	3,4	7,0	1,0	115	Leccino
	406	2,9	19,2	22,1	6,8	169	Gentile
	416	0,7	0,5	1,3	0,7	305	Picholine
	419	0,4	1,1	1,5	3,9	633	Bianchera
2002	32	3,1	20,9	24,1	6,9	396	Dritta
	41	3,9	18,2	22,1	4,7	189	Gentile
	44	3,5	4,8	8,3	1,4	131	Leccino
	91	7,8	23,9	31,7	3,1	370	Bianchera
	92	3,7	2,2	5,9	0,6	215	Picholine

In fact, both phenolic substances were found in oil samples obtained from the olives *cv.* mentioned above. It is interesting to observe that the quantities of (+)-pinoresinol and (+)-1-acetoxypinoresinol vary in relation to the *cv.* In particular, the relation A/P is characteristic for each of the *cv.* examined.

Additionally, table 2 highlights that the samples with the highest relative value to this relation were sample 322 for the year 2001, belonging to *cultivar* Dritta and 406 concerning *cultivar* Gentile; whereas for the year 2002 it was again sample 32, concerning *cultivar* Dritta and sample 41 concerning *cultivar* Gentile.

Relatively high values of this relation were also found for the year 2001 in sample 419 and, for the year 2002, in sample 91, both belonging to the *cultivar* Bianchera. But in samples 416 of 2001 and 92 of 2002, both concerning *cv.* Picholine, values of 0.7 and 0.6, were found, which is very low even if very similar.

It seems that lignans do not endure significant variations with the preservation process; in fact, one can see by comparing the two years, the content is almost constant.

Along with lignans, the total polyphenols content was examined, as showed in table 2. One can observe that for 2001 it is the 419, concerning

cultivar Bianchera, the sample with the highest value of PPT, followed by sample 322, regarding *cultivar* Dritta.

Other than these two samples, there is a considerably high value for the year 2001 in sample 416, concerning *cultivar* Picholine and, for 2002 the sample 32 concerning *cultivar* Dritta, followed by sample 91 concerning *cultivar* Bianchera.

Chlorophylls

The results of determinations in HPLC are showed in table 3, whereas Diagram 1 represents a typical chromatogram HPLC.

Table 3. Pigments (mg/kg oil) analysed varieties: averages (2001-2002) for geographical areas

Tablica 3. Pigmenti (mg/kg ulja) analiziranih ulja: prosjeci (2001-2002) za geografska područja

Comparison by pigments

	Chlb	Chla	Pheob	Pheoa	Violaxanthin	Lutein	Neoxanthin	Σ pigments
Abruzzo	0,87	0,06	0,80	9,84	0,42	7,72	0,78	20,49
Croatia	4,46	1,95	1,29	29,28	2,22	13,81	2,27	55,27

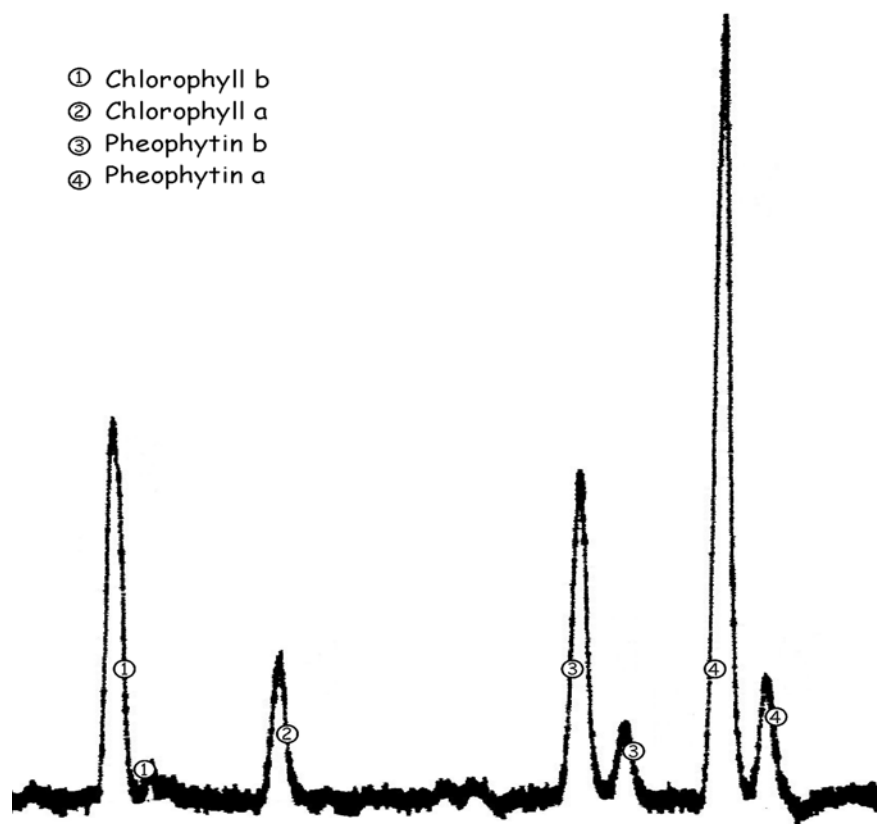
The HPLC results analysis are appropriate for the study of olive oil pigments in terms of separation of different categories and quantitative determination of single compounds: the records obtained highlight the opportunity of a systematic and comprehensive research of the complete fraction, in order to achieve a more complete characterization of productions, in particular of the monovarietal ones.

These records essentially agree with parallel measures carried out in other Mediterranean countries, both for what concerns the order of size and in terms of relative quantities. (M.I. Minguez-Mosquera, 1992), (E. Psomiadou, M. Tsimidou, 2001), (B. Gandul *et al.*, 1996), (M.I. Minguez *et al.*, 1990), (M.I. Minguez *et al.*, 1989).

The varietal comparison (Table 3) has provided interesting results: the Istrian production, in general, shows higher levels of pigments compared to those of central Italy. Considerable differences can be noticed even in relation to the single varieties of the distributional areas considered.

Diagram 1. Chromatogram HPLC of pigments of a variety oil Bianchera

Diagram 1. HPLC kromatogram pigmenata kultivara ulja Bjelica

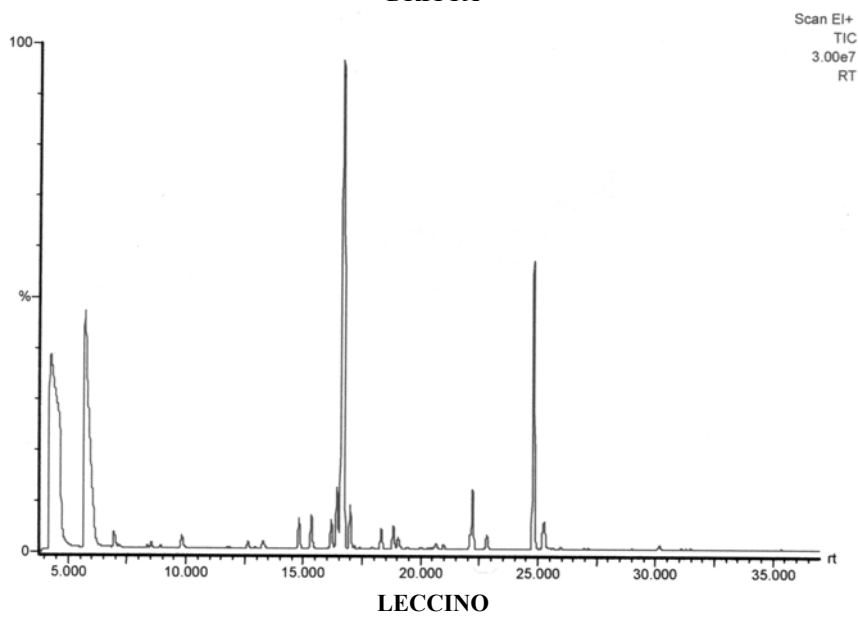
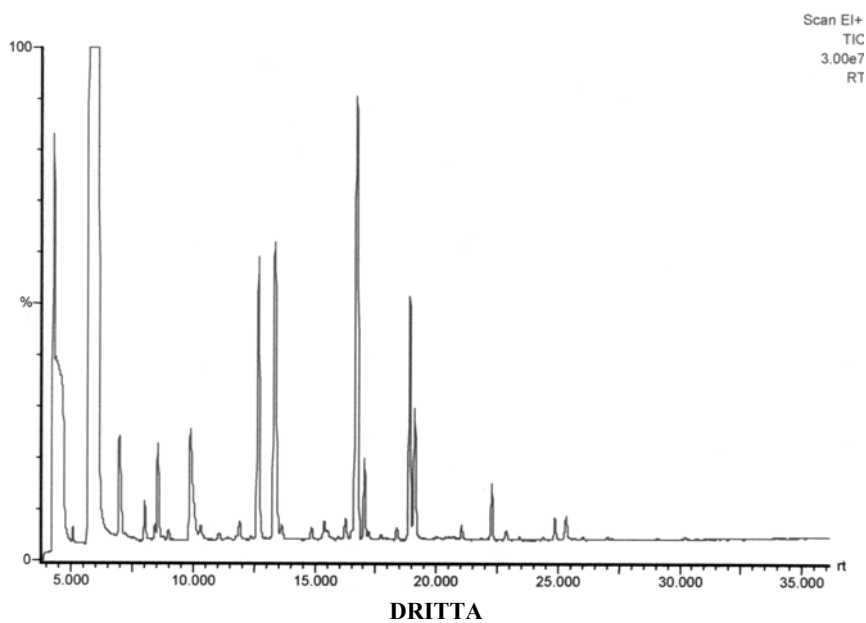


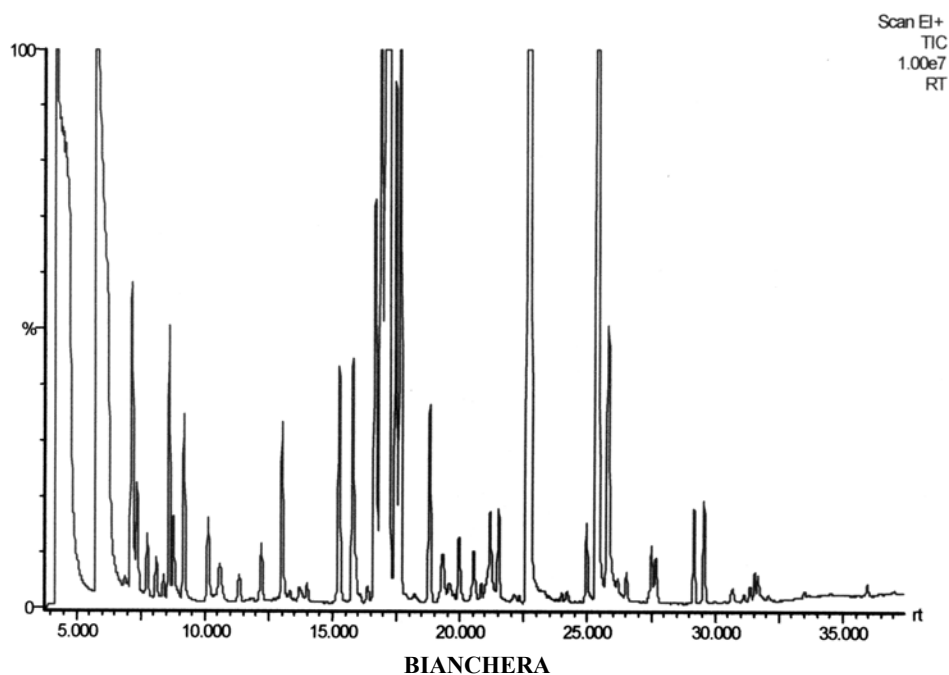
Volatile compounds

The analytic method used in this research (DHS-GC-MS), has allowed the retrieval and the identification of more than 70 substances forming the fragrance of the examined product. It is important to emphasize that these substances correspond exactly to the ones detected by our sense of smell. In fact, methodology does not expect pre concentration stages on polymeric or carbonaceous matrix and therefore it tends to eliminate possible valuation mistakes due to an incomplete release of analytes of interest or to the formation of degraded or adulterated products. Diagram 2 reports the chromatograms concerning the leading position of 3 extra virgin olive oils of typical varieties from Abruzzo, Dritta e Leccino, and a Croatian variety, the Bianchera.

Diagram 2. Aromagram of oil of the variety Dritta, Leccino e Bianchera.

Diagram 2. Aromagram ulja kultivara Dritta, Leccino i Bjelica





Some compounds content of the “flavour” are fairly interesting, mainly aldehyds, alcohols and esters, as already highlighted by previous researches, occur with extreme variability (Morales M.T. *et al.*, 1995), (Flores M. *et al.*, 1997), (Angerosa F. *et al.*, 1999).

Sensible percentage differences are revealed in the concentration of the volatile fraction of the extra virgin analysed; a significant weight can be given to the variety of the raw material, even though this weight can appear extremely conditioned by the agronomic and harvesting processes and by the type of extraction and the concerning operational techniques.

The limited sampling does not allow one to reach, inside the single cultivar, the definition of an objective profile in function with the agronomic and technological processes.

The differences found in the fragrance concentration of the analysed samples, though within the limits of the work in progress in the initial stage, assuming the use of the method we applied both in the definition of the variable “finger print” and in the individuation of the quality index in function with agronomic and technological variables.

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Primjeno - Received:

14. 02. 2006.