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The Influence of Environmental Factors on Polyphenol and Tocopherol Content in Extra Virgin Olive Oil

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***Abstract* – The two-year study was carried out using samples of extra virgin olive oil obtained from 5 autochthonous genotypes. Cultivars studied represent the most important varieties of Berat and Tirana area. *Boç*- located mainly in Tirana and Durrës area is adapted to poor and slope soils, but less tolerant to low temperature; sensible to the olive fly (*Bactrocera (Dacus) oleae)*. *Marks* cultivar is diffused mainly in Berat and Fier. It is tolerant to *Spilocaeaoleagina*, the causal agent of peacock leaf spot and *Pseudomonas syringae subsps. Savastanoi* olive knot but highly susceptible to olive fly (*Bactrocera (Dacus) oleae*). *Ulli i Bardhë Pobrat*- originated possibly from "white olives" of central Albania for biomorphologic and agronomic characteristics they have in common. It is diffused in the areas through Fier and Berat where it consists in 50-60 % of total surface planted in olive trees. *Ulli i Bardhë Tiranë*- originated in Tirana area, latter diffused in central Albania, mainly in Tirana, Durrës, and Krujë. It is also found in Lezhë and Shkodër where it is known by the same name; tolerant to biotic and abiotic stress. It is adapted to prolonged summer, drought and cold. *Ulli i Zi Tiranë*- diffused in olive groves of Tirana; tolerant to biotic and abiotic stress and has good production in the slope soils. Shows good tolerance to prolonged summer, drought and cold stress. Resistant to peacock leaf spot (*Spilocaeaoleagina*). Olive samples were collected at origin location in two different regions of Albania, harvested and then processed within 24h at the mini oil mill "Oliomio" on a two-phase extraction process (processing capacity of 30-50 kg/ hour). The olive harvest period was from November to December 2012-2013 and 2014-2015. Phenol and tocopherol content as well as their organoleptic analysis was carried out. The results show the influence of environmental factors on the chemical and organoleptic quality of extra virgin olive oil.**

***Keywords* – Autochthonous Cultivar, Extra Virgin Olive Oil, Polyphenols, Tocopherols.**

I. INTRODUCTION

In order to achieve the objective of optimizing olive production, the regeneration, characterization and utilization of olive genetic resources is a very important factor. To enhance and promote the production of high quality olive, most influential factors must be taken into consideration. Recognition of these factors can lead to quality and high standard product. Furthermore, for good and sustainable agricultural practices of production, the influence of environmental and varietal factors such as the period of maturation, the processing technology as well as oil storage conditions play an essential role. Particular emphasis is given to the degree of infection of olives, especially from the olive fly (*Bactroceraoleae*, Gmel) in the chemical composition of oil produced [1][2]. Oils obtained from infected olives contain lower amount of total phenol and therefore lower stability. The quality of virgin olive oil is significantly influenced by the presence of polar phenolic compounds [3][4][5][6]. The importance of polyphenols in the stability and quality of olive oil supports the efforts of researchers to develop adequate technology [7][8][9][10][11].

II. MATERIALS AND METHODS

The study was conducted using samples of extra virgin olive oil obtained from 5 Albanian genotypes. Analysis of antioxidant compounds (phenol and tocopherol factions) of monocultivar autochthonous oils were performed at "G. D'Annunzio" University Laboratory, Chieti-Pescara Italy. Data resulting from analytical analysis confirmed that all samples belonged to the category of extra virgin olive oil. Factors affecting the quality of the oil were also assesed. EVOO samples (5) were collected from olive fruits harvested in November 2012 to 2013 and 2013 to 2014 at middle ripening stage, respective oils were immediately obtained. Olives were collected at origin location in two important cultivation areas: Tufinë -Berat and Pobrat-Tiranë.

Oil extraction process took place at the mini oil mill "Oliomio", "Toscana Enologica Mori" (Picture 1). After careful cleaning and washing the olives started processing (Picture 2). The oil mill was washed and cleaned after each cycle. The paste obtained from each sample was futher processed for 30 minutes in a temperature 20±0.3°C.

The following analysis were carried out:

Fatty acid analysis using methyl ester from CGC by WW Christie Hourly 1998.

Total phenolic compounds by Singleton 1965 and Cichelli 1999.

Tocopherol Fraction by IUPAC, 1988

III. RESULTS

A. Polyphenol & Tocopherol Content

The extraction method is performed in such a way as to obtain the total amount of polyphenols and tocopherols and maintain the quality of the product. These compounds not only affect the sensory quality of olives and olive oil, but are also important for determining the specificity, biodiversity and nutrition quality of this product.

B. Polyphenol Content in Olive Fruit

The main phenolic compound in olive fruit is oleuropein, a heterosidic ester of elenolic acid with 3,4 dihydroxyphenethylalcohol (hydroxytyrosol, HYTY). Other glycosides found in the fruit are ligstroside, a heterosidic ester of elenolic acid with 4-dihydroxyphenethylalcohol (tyrosol, TY) and

verbascoside, a heterosidic ester of caffeic acid with hydroxytyrosol. Flavones and flavonols have also been reported. Green olives contain a significant amount of total polyphenols (>1,000 ppm expressed as caffeic acid) which is reduced during alkali treatment or brine fermentation [12]. Advances in research on the evolution of phenolic compounds in olives during growth and maturation have provided useful information on the fate of main glycosides of olive as oleuropein and verbascoside. The oleuropein content can reach 14% of the dry matter in young fruit and remain very important at harvest for green picked varieties. In general, small-fruit varieties are characterized by high oleuropein and low verbascoside contents, while large fruit varieties are characterised by low oleuropein and high verbascoside [13][14].

Oleuropein and demethyloleoropein stimulate ovipositioning of *Dacus oleae*. Degradation products from the hydrolysis of oleuropein have been found to inhibit ovipositioning of *D. oleae*, a fact attributable to the formation of 3,4-dihydroxyphenethylalcohol [15]. The chemical relationship between oleuropein, elenolic acid glycoside and demethyloleoropein and their respective levels during fruit maturation suggests that they may be related biosynthetically. One possibility is that the last 2 components are formed from oleuropein by the action of esterases.

Polyphenols are significantly related to the quality of virgin olive oil and their contribution to the oxidative stability of the oil is widely accepted. The impact of the recent findings on the nutritional role of these components is important for the agro-food industry. Good Manufacturing Practices and decisions about the cultivation of certain olive varieties should take the factor of "polyphenols" into consideration. However, enrichment of an olive oil with phenolic components has limitations due to their contribution to the sensory quality of the oil.

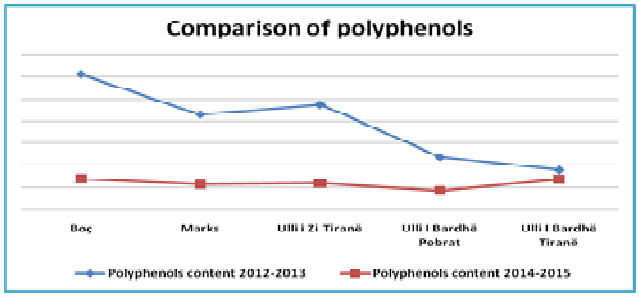
More bitter olive oils may not be acceptable to customers even if they contain nutritional components or have long shelf lives. It seems possible that in the near future polyphenols may prove to be the key component for evaluating the overall quality of virgin olive oil.

Fig. 1. Cultivars in the study


Fig. 2. Oil mill. The process of olive washing and cleaning

Table 1. Polyphenol Content in the First 2 Years of Study.

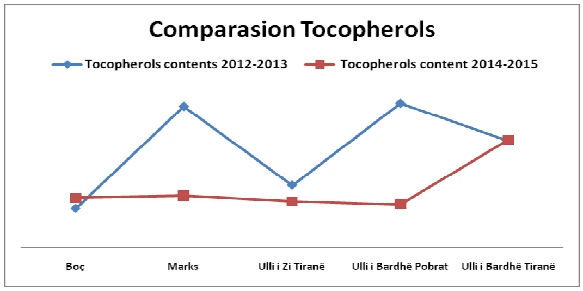
Variety	Polyphenols content 2012-2013	Polyphenols content 2014-2015
Boç	306	70.9
Marks	215	57.9
Ulli i ZiTiranë	238	60.0
Ulli i Bardhë Pobrat	119	43.4
Ulli i Bardhë Tiranë	90	69.4



Graph. 1. Comparison of Polyphenols

Table 2. Tocopherol Content in the First 2 Years of Study.

Variety	Tocopherols content 2012-2013	Tocopherols content 2014-2015
Boç	75.5	96.0
Marks	270	100
Ulli i Zi Tiranë	119.9	89.0
Ulli i Bardhë Pobrat	276.1	83.1
Ulli i Bardhë Tiranë	204.7	207



Graph 2. Comparison of Tocopherols

Comparison of the two indicators over the two years of study (Graphic 1, 2; Table 1, 2) show there are significant differences in their values, which provides useful information over the factors that affect the quality of olive oil. Temperature, humidity and dynamics of olive fly infection were the main environmental factors influencing the variability of indicators.

Monocultivar autochthonous extra virgin oils appear with their bitterness profiles.

Albanian consumers must recognize all features and quality of oils they want to consume. Bitterness can result from a wide range of molecules with varying size and functional groups. The ability to predict bitterness from molecular composition is rather limited. Bitterness is

perceived primarily at the back of the tongue. Astringency is a sensation of dry mouth associated with the contraction of tissues. Astringency is produced by the interaction of phenols with proteins in the saliva.

Certification of oil through physical-chemical analysis and organoleptic tests means security and guarantee for quality. In the context of harmonization of laws, EU Regulation (CE) n.2568/91 is followed.

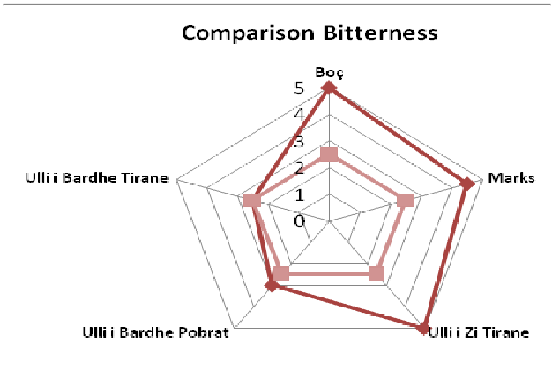
Sensory analysis of olive oils was carried out from the National Panel of olive oil composed of 8-10 experts. Samples of autochthonous monocultivar olive oils were collected at origin location: in Tufinë Tirana-cv Ulliri i Zi, i Bardhë and Boc; in Pobrat Berat-Marks and Ulliri i Bardhë Pobrat.

Monocultivar oils were analysed according to the Method COI / T.20 / Doc. N. 15 / Rev 2; COI / T.20 / Doc.5 / Rev 1; COI / T.20 / Doc.6 / Rev 1; COI / T.20 / Doc. 15 / Rev. 4. The data were statistically analysed according to COI / T.20 / Doc. nº 22 Annex 3.
Table 3 shows 5 cultivars who presented interest in the bitterness indicator of their organoleptic profile.

Table 3. Cultivars Present in the Study

Cultivar	Organoleptic features 2012-2013	Organoleptic features 2014-2015
Boc	5; bitter, sharp	2.5; sweet, woody, apple
Marks	4.5; sharp, slightly fruity, varietal, bitter	2.5; sweet, woody, apple
Ulli i Zi Tiranë	5; highly sharp, fruity, varietal, slightly bitter, green leaves	2.5; sweet, woody, apple
Ulli i Bardhë Pobrat	3; sweet, woody, apple	2.5; sweet, woody, apple
Ulli i Bardhë Tiranë	2.5; sweet, woody, apple	2.5; sweet, woody, apple

As seen from the Graphic 3, bitterness intensity is higher in the year 2012-2013 and therefore correlated to polyphenol values.



Graph 3. Bitterness comparison

IV. Conclusion

From the results can be concluded that for extra virgin olive oils of autochthonous varieties at their origin location, harvested in the same period, cultivated during two years of study, processed at the same oil mill, it is clearly visible the influence of environmental factors in polyphenol content and therefore in their sensory quality and bitterness.

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She was born in Tirana, Albania on 27 March 1970. She graduated from University of Tirana, Faculty of Natural Sciences in Chemistry in 1998. She received a Postgraduate Diploma from CIHEAM-IAMZ in "Olive growing and oil technology", University of Cordoba, Department of Agronomy, Spain in 2002 and defended her Phd thesis "Use of "*in vitro*" method for the multiplication and sanitation of fruit trees rootstock and olive in our country" in 2008.

After receiving her education, during the years 1998-2000 she worked as chemist at the University "Ismail Qemali" Vlore Chemistry Laboratory. In 2000 started working as researcher at ATTC (Agriculture Technology Transfer Center), former Institute of Arboriculture. Since 2008 holds the position of Official Head of the National Olive Oil Panel Test in Albania. She has extensive experience in "*in vitro*" cultivation methods as well as in Oliviculture, following several courses in IAM Bari and Department of Plant Protection and Applied Microbiology, University of Bari, Italy.