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Quantitative Determination of Selected Volatile Compounds in Extravirgin Olive Oils From Tuscan Olive Germoplasm by SPME/GC-MS

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Summary: *A quantitative method, based on spme gc-ms, for the quantification of olive oil selected volatile compounds was developed. The method was used to study the variation within the olive oils from the different genotypes of Tuscan olive trees conserved at "Santa Paolina" experimental farm (Follonica, Italy).*

Keywords: *extravirgin olive oil, germplasm, solid phase micro-extraction*

Introduction

Italy possesses one of the widest olive genetic assets in the world but a very limited number of cultivars are used intensively. The majority of the olive germplasm is at risk of genetic erosion because abandoned and only marginally cultivated. There is an increasing interest in the production of high-quality olive oils as well as monocultivar olive oils linked to limited production areas. The "Santa Paolina" experimental farm in Follonica is a centre for plant biodiversity conservation maintaining about 1000 accessions of olive tree 82 of them collected within the Tuscany region. While trees are well characterised for morphological aspects [1] and in some cases for genetic diversity [2], the produced oils or that can be produced, by these varieties have been only partially characterised.

In order to collect information on the characteristics of the oils related to Tuscan accessions, 130 mono-cultivar EVOOs from 67 genotypes were produced over two years.

In this contribution, we present a developed method based on SPME GC-MS to quantitatively measure a selection of volatile compounds in the analysed olive oils. The method was then applied to study the variation within the olive oils from the different genotypes.

Experimental

Fruits. During the autumn 2010 and 2013 along a two-month period, all the cultivars which presented enough fructification for the production of the oil were harvested following the stage of ripening. All the fruits were hand harvested in the morning and processed in the same afternoon to avoid possible fermentations or any other alteration of the final product. Forty kilos of healthy fruits were harvested for each 4-plants group.

Oils. Immediately after harvesting, the olives were washed and crushed by a two-phase Oliomio® continuous mill (Toscana Enologica Mori, Tavarnelle V.P.,

Italy). The system reproduces, at a small scale, the industrial method of oil extraction so that the resulting EVOOs were as much as possible similar to those produced in an industrial plant. Prior to the analysis, oils, protected from the light, were kept at 12 °C for 3 months.

Volatile compounds. A 3 g of oil was placed in a 20 ml glass vial, spiked with 50 µl of a solution of 4-methyl-2-pentanol (Aldrich, Milan, Italy) as internal standard, capped and housed in the auto-sampler (CTC Analysis AG, Zwingen, Switzerland).Volatile compounds were extracted on a fused silica fiber (2 cm), coated with DBV/CAR/PDMS 50/30 µm (SUPELCO Bellefonte, USA) and then desorbed at 250 °C in the injector port of a GC coupled to a mass detector which operates in electron ionization mode (EI; 70 eV) with a scan range m/z 40–300 (GC Clarus 500, PerkinElmer, Norwalk CT, USA). Separation was achieved on a HP-Innowax fused-silica capillary column (30 m, 0.32 mm ID, 0.5 µm film thickness; Agilent Technologies, Palo Alto, CA, USA). The GC oven temperature program consisted in 50 °C for 5 min, 50-250 °C at 5 °C·min^{-1}, 250 °C for 1 min. He was used as carrier gas (2 ml·min^{-1}). Calibration curves were prepared for 18 volatile compounds: (2Z)-2-penten-1ol; (E)-2-hexen-1-ol; (E)-2-hexenal; (E)-2-pentenal; (Z)-3-hexen-1-ol; (Z)-3-hexenyl acetate; 1-penten-3-ol; 1-penten-3-one; hexanal; hexyl acetate; hexanol; 3-methyl-1-butanol; β-ocimene; 2-phenylethanol; ethyl acetate; ethyl benzene; hexanoic acid; pentanal (Sigma–Aldrich).

Results

The calibration curves obtained for each volatile compound and used for the quantification in the oils are summarized in Table 1.

Table 1. List of calibration curves. Range of concentration tested over 5 points, linearity of the curves and sensitivity (slope).

	range (µg/kg)		linearity	
Compound	**min**	**max**	**R^2**	**slope**
(E)-2-Hexenal	24	5590	0.990	0.266
(E)-2-Hexenol	17	803	0.990	0.198
(E)-2-Pentenal	21	9693	0.985	0.348
(Z)-2-Pentenol	15	3614	0.992	0.290
(Z)-3-Hexen-1-ol	34	17305	0.992	0.385
(Z)-3-Hexenyl acetate	30	6967	0.988	0.358
1-Penten-3-ol	16	7546	0.988	0.365
1-Penten-3-one	24	11035	0.996	0.485
3-Methyl-1-butanol	25	5938	0.993	0.413
Acetic Acid	22	5184	0.991	0.132
β -(E)-Ocimene	28	1316	0.996	0.009
β -Phenylethanol	43	11025	0.995	0.058
Ethyl acetate	56	29046	0.997	0.719
Ethyl benzene	24	11361	0.994	0.693
Hexanal	33	17252	0.978	0.034
Hexanoic acid	27	6272	0.971	0.043
Hexyl acetate	37	9471	0.995	0.188
n-Hexanol	22	11342	0.994	0.357
Pentanal	56	29046	0.996	0.044

As expected, there was a great variability among the different varieties and between the 2 years of production. Considering the sum of all the volatile compounds quantified, the variation between the 2 years was on average within 10% while considering each cultivar the variation between the 2 seasons varied from 4 to 99%. The major variations between the 2 seasons were observed for (Z)-3-Hexen-1-ol that increased (from 2011 to 2013) on average by 273 times and for (E)-2-pentenal decreased (from 2011 to 2013) of 26 times.

The most abundant compounds quantified ere hexanal (1286 - 32636 µg/kg), β-ocimene (63 - 69970 µg/kg), 1-penten-3-one (110 – 3758 µg/kg) and (Z)-3-hexenyl acetate (n.d. – 6027 µg/kg) with strong differences among cultivars.

Conclusions

This study indicates the high variability of volatile compounds associated with the different Tuscany olive varieties maintained at the "Santa Paolina" collection. Since all the trees were harvested in the same place under the similar agronomic conditions and the oils were prepared in steady conditions, a significant part of the variability observed in the volatile compounds can be associated with genetic characteristics. These data together to fatty acids, polyphenols, and sensory data will be used to deeply characterize the whole Tuscan olive germplasm aiming to exploit monocultivar olive oils production as well as to select plants to be used in further breeding programs.

References

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