CORRELATION OF OLEOCANTHAL AND OLEACEIN CONCENTRATION WITH PUNGENCY AND BITTERNESS IN 'KORONEIKI' VIRGIN OLIVE OIL

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**Correlation of Oleocanthal and Oleacein Concentration with Pungency and Bitterness in cv. Koroneiki Virgin Olive Oil**

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**Abstract**

It is known that the organoleptic attributes of pungency and bitterness in olive oil are attributed to phenolic compounds. Recent research has demonstrated that oleocanthal, a secoiridoid phenolic, is a naturally occurring anti-inflammatory and neuroprotective agent, and that it elicits a throat stinging sensation through a specific TRPA1 receptor. Oleacein, a hydroxytyrosol derivative is considered as the most powerful antioxidant in olive oil and although it differs from oleocanthal only by one additional phenolic hydroxyl, organoleptically it is associated more with bitterness and less with pungency. The present study examined the correlations between oleocanthal and oleacein concentrations and the intensity of pungent and bitter sensations in a group of 21 virgin olive oil samples of the Koroneiki variety, produced in a specific geographic region of Greece (Messini). The qualitative and quantitative determination of the chemical compounds was carried out by a new method based on quantitative 1H-NMR in CDCl3 at 600 MHz after extraction of olive oil with acetonitrile. The concentrations of the studied compounds ranged for oleocanthal between 34.6-154.6 mg/L (mean value 94.5±31.8 mg/L) and for oleacein between 11.5-191.9 mg/L (mean value 63.3±36.1 mg/L). The organoleptic assessment of the samples was performed by a trained sensory panel according to the IOC method. The values of the positive attributes were distributed between 0.40-2.65 (mean value 1.45±0.72) for bitterness, 1.50-3.70 (mean value 2.62±0.56) for pungency and 0.40-3.60 (mean value 2.14±0.82) for fruitiness. The results showed that there is a positive correlation ($r=0.79$, $p≤0.05$) between the concentration of oleocanthal and oleacein, and the intensity of pungency and bitterness, suggesting that virgin olive oils with intense organoleptic attributes may offer more health benefits. Moreover, a proposal for a new index for describing and quantifying the positive organoleptic attributes of virgin olive oils is discussed.
INTRODUCTION

Organoleptic attributes are major determiners of the quality of olive oil (Gomes da Silva et al., 2012). Extra virgin olive oil (EVOO), the pillar of the Mediterranean diet, is characterized by three positive organoleptic properties: pungency, bitterness and fruitiness. According to the official organoleptic descriptors of the International Olive Council (IOC), the sense of pungency is perceived throughout the mouth cavity and in particular in the throat. Bitterness is perceived in the circumvallate papillae on the “V” region of the tongue while fruitiness through the back of the nose (COI/T.20/Doc. No 15/Rev. 4).

The perception of pungency and bitterness in the oropharyngeal region of the oral cavity is due to specific phenolic compounds associated with each of these organoleptic attributes. Oleocanthal (decarboxymethyl ligstroside aglycone or p-HPEA-EDA) elicits a stinging sensation felt at the throat. In fact, research has demonstrated that TRPA1, a sensory receptor which is found in the oropharyngeal region triggers the irritant attributes of oleocanthal (Peyrot des Gachons et al., 2011). The bitter taste of olive oil is also related to the presence of phenolic compounds, one of which is oleacein (3,4-dihydroxyphenylethanol-elenolic acid dialdehyd or 3,4-DHPEA-EDA) (Andrewes et al., 2003; Czerwińska et al., 2012; Gutierrez-Rosas et al., 2003; Inarejos-García, 2010). Besides their gustatory associations, these particular phenolic compounds have significant health benefits. Oleocanthal is a powerful non-steroidal anti-inflammatory agent, which is similar to ibuprofen, and which may have therapeutic benefits against neuro-degenerative diseases, joint-degenerative diseases and cancer (Cicerale et al., 2009 & 2012). Similarly, oleacein displays anti-breast cancer properties (Menendez, 2008), possesses considerable antioxidant benefits (Pavia-Martins, et al., 2009), and protects against cardiovascular diseases (Czerwińska et al., 2012).

Parameters that can affect the organoleptic attributes and phenolic compounds of olive oil are associated with olive tree cultivar (Olea europaea L.), geographic region, agronomic practices, environmental factors, ripeness index, extraction techniques, storage conditions and domestic heat application (Bendini et al., 2012). Considerably different oleocanthal concentrations in EVOOs depending on the cultivar and area have been reported: 8.3 mg/kg in EVOOs produced from the Taggiasca (Franconi et al., 2006), 21.0 mg/kg from Oliarola (De Stefano et al., 1999), 43.8 mg/kg from Frantoio (Serrili et al., 2007), 53.0 mg/kg from Seggianese (Franconi et al., 2006), 87.9 mg/kg from Cornicarba (Inarejos-Garcia et al., 2010) and 78.2-92.8 mg/kg from Coratina (De Stefano et al., 1999; Serrili et al., 2007). Especially for Koroneiki EVOOs, it has been found that oleocanthal concentrations can range from 62.6 mg/kg (Allalout, et al., 2009) to 76.8 mg/kg (Stefanoudaki et al., 2011). EVOOs produced in U.S.A. contained a lower oleocanthal concentration than in Italy (Beauchamp et al., 2005).

Rain-fed olive trees produce EVOOs containing higher oleocanthal concentration compared to irrigated ones (Gomez-Rico et al., 2006). Premature picking date of olive fruits leads to EVOOs with increased oleocanthal concentration (Morello et al., 2004; Gomez-Rico et al., 2006). Oleocanthal concentration in EVOOs decreases relative to storage period and exposure time to oxygen and light (Cicerale et al., 2011). Although phenolic content is generally reduced by heating during cooking, oleocanthal displays remarkable stability especially when its initial concentration in EVOO is high (Gomez-Alonso et al., 2003; Allouche et al., 2007).

Oleacein content in EVOOs also differs depending on cultivar. Arbequina EVOO contained 68.3 mg/kg oleacein, Arbosana EVOO had a concentration of 30.1 mg/kg (Allalout, et al., 2009) whereas EVOO obtained from the Coratina variety contained 253.9 mg/kg (Stefanoudaki et al., 2011). In olive oil from the Koroneiki
cultivar, oleacein concentrations ranged from 69.6 mg/kg (Allalout, et al., 2009) to 76.8 (Stefanoudaki et al., 2011). In contrast to oleocanthal but under particular irrigation practices, oleacein content in Aberquinn trees was noted to reach 780 mg/kg (Tovar et al, 2001). Oleacein decreases during storage (Esti et al, 2009) and is not as stable as oleocanthal during the cooking process (Gomez-Alonso et al., 2003).

The aim of this study was to further our knowledge of the relationship between the concentration of oleocanthal and oleacein and the intensity of pungency and bitterness in the Koroneiki cultivar. Therefore, we quantified the concentration of oleocanthal and oleacein in olive oil samples of this cultivar and conducted an evaluation of the organoleptic attributes of the samples by means of a panel test. Moreover, we correlated the relationship between the concentration of these phenolic compounds with the intensity of pungency and bitterness in order to suggest an index for describing and quantifying the positive organoleptic attributes of olive oil.

MATERIALS AND METHODS

Olive Oil Samples

A group of 21 EVOO samples produced in a specific geographic region of Greece (Peloponnese, Messini, lat. 37° 3’ 4N, long. 22° 0’ 28E) were studied. All samples were obtained from orchards of rain-fed olive trees of the Koroneiki variety and harvested in the early maturation stage during the period November -December 2011. The olive fruits were immediately transferred and extracted in local olive oil mills using two-phase centrifuge systems. The olive oil was stored at the mills, in local cooperatives or at packing plants under appropriate conditions.

Oleocanthal and Oleacein Determination

For oleocanthal and oleacein extraction, 5 ml of olive oil were mixed with 20 ml of cyclohexane and 25 ml of acetonitrile. The mixture was homogenized using a vortex mixer for 30 sec and centrifuged at 1,500 rpm for 2 min. Acetonitrile phase was collected, 1 ml of a 1.0 mg/ml syringaldehyde solution in acetonitrile was added and the mixture was evaporated under reduced pressure (Buchi rotary evaporator). The residue was dissolved in 0.6 μl of CDCl₃ and transferred to a 5 mm NMR tube. One-dimensional spectra were recorded at 600MHz using a NMR spectrometer (Bruker Avance-600). Calibration curves were prepared using pure oleocanthal or oleacein isolated from olive oil extracts, by addition of standard compounds in a selected olive oil naturally free of oleocanthal and oleacein. The quantification was based on the integration ratio between the aldehyde proton of syringaldehyde and the aldehydic protons of oleocanthal or oleacein appearing between 9.17 and 9.30 ppm.

Sensory evaluation

The EVOO samples were evaluated by 10 panel members of the Olive Oil Taste Panel of the Technological Educational Institute (TEI) of Kalamata according to the European Commission (EC Reg. 640/2008) and IOC regulations (IOC/T.20/Doc. No 15/Rev.3). The panel members have more than four years experience in evaluating olive oil. The intensity of the positive attributes, fruity, pungent and bitter, and the defects were expressed as median scores. The classification of olive oil as extra virgin requires that the median of the defects is 0 and the median for fruity is above 0 (EC Reg. 640/2008).
RESULTS AND DISCUSSION

In the samples studied, oleocanthal concentrations were between 34.6-154.6 mg/L with a mean value 94.5±31.8 mg/L. These values are in accordance with data of other studies (Stefanoudaki et al., 2011) despite measured using a different methodology, and classify the Koroneiki variety among those with the highest oleocanthal concentration, i.e., with Cornicarba and Coratina (Inarejos-Garcia et al., 2010; De Stefano et al., 1999; Servili et al., 2007). The high level of oleocanthal may have been influenced by the cultivation practices that increase concentrations, in particular, rain-fed conditions, premature picking date of harvesting, rapid elision as well as short (2-3 months) storage period prior to sampling and evaluation.

Oleacein concentrations were between 11.5-191.9 mg/L with a mean value 63.3±36.1 mg/L. These data are in agreement with those reported for the Koroneiki cultivar by other studies, although in some cases trees were grown under irrigated conditions (Allalout, et al., 2009; Stefanoudaki et al., 2011).

The sensory evaluation of samples showed that overall positive attributes were distributed between 0.40-2.65 with a mean value 1.45±0.72 for bitterness, 1.50-3.70 with a mean value 2.62±0.56 for pungency and 0.40-3.60 with a mean value 2.14±0.82 for fruitiness. Based on the above, EVOOs from the studied region are characterized as light and well balanced, which means that the median value of all positive attributes were less than 3 points while the medians of pungency and bitterness does not exceed the median value of fruitiness more than 2 points (IOC/T.20/Doc. No 15/Rev.3).

It has been reported that pungency and bitterness intensity are correlated with oleocanthal and oleacein concentration respectively (Esti et al, 2009). The present study has shown that the concentration of oleocanthal correlated with both pungency (r=0.57, p≤0.05) and even more with bitterness (r = 0.73, p ≤ 0.05). Simultaneously, oleacein concentration showed a similar correlation with bitterness (r=0.68, p≤0.05) but to a lesser degree with pungency (r=0.60, p≤0.05). The above suggests that both oleocanthal and oleacein are correlated with both bitterness and pungency in EVOOs.

A strong correlation (r=0.79, p≤0.05) was observed between the sum of oleocanthal and oleacein concentrations with the corresponding sum of bitterness and pungency (Figure 1). Based on this figure, it is proposed that the sum of oleocanthal and oleacein concentrations could be used as an indicative index for the sensory evaluation of olive oil when the latter is not feasible, and conversely, the corresponding sum of bitterness and pungency intensities as an indicator of the antioxidant effect, if chemical analysis is not available. Necessarily, further investigation is needed for the calibration of this index.

Literature cited


Fig. 1. Correlation between the sum of bitterness and pungency intensity and the sum of oleocanthal and oleacein concentration.