Kefalonian Olive Oils:Oleocanthal and Oleacin Abundance and Antioxidant Activity

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Introduction

Olive oil is a key component of the Mediterranean diet which is associated with a lower incidence of certain diseases. These health-promoting properties have been partially correlated to the oil's high levels of monounsaturated fatty acids (mostly oleic acid) and the presence of antioxidants. The antioxidant substances of olive oil include Vitamin E (alpha-tocopherol), carotenoids and phenolic compounds both simple phenols such as hydroxytyrosol and complex phenols such as oleuropein (OL). Phenolic compounds in general and OL derivatives in particular can act as natural antioxidant in various ways, thus becoming important molecules both for the food stability and the human health. Among phenolic compounds, oleocanthal and oleacein, have recently gained wide attention. These compounds are endowed with antimicrobial, anticancer, and hypoglycemic effects, and are considered key oxidation inhibitors. Oleacein has been declared a more potent antioxidant than hydroxytyrosol. Furthermore, the interest in these derivatives has been enhanced because of their reported antiinflammatory properties, as oleocanthal has shown intense antiinflammatory effects comparable to ibuprofen [1]. Recently, oleocanthal has also been proposed as a promising agent to induce selectively cancer cell death via lysosomal membrane permeabilization [2]. In 2012, the European Union (EU) made a health claim labelling regulation 432-2012. In this regulation, it is stated that olive oils with polyphenols over 250 mg/kg can put a health claim on the label as it reduces LDL oxidation.

Some phenolic compounds tend to maintain themselves over time better than others. Many factors affect the phenolic levels, including the olive variety, geographic origin, the type of mill, time of harvest and bottling, the type of bottle used, the amount of time spent on the shelf, and conditions in the warehouse or in transport.

Objective

The island of Kefalonia has native cultivars of olive trees, some of which are over 1500 years old.

The aim of this work was to examine the dependence of the olive oil oleocanthal and oleacein concentration and antioxidant activity on the olive cultivar.

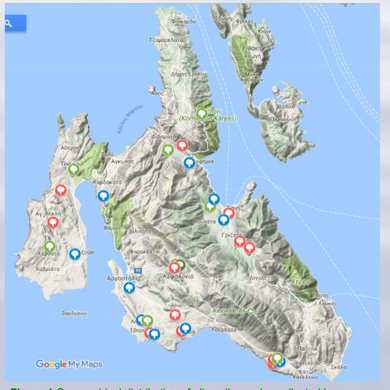

Figure 1. Geographical distribution of olive oil samples collected in Kefalonia island, Greece

Methods

Olives were collected early in the harvest period (October), so that the oil produced would be of the extra-virgin category. Only healthy and undamaged fruits were processed, all under the same conditions.

Olive samples consisting of 3 kg each were transported to the lab. Olives were crushed, pitted, blended and homogenized for 45 min. Care was taken so that the temperature during homogenization was always below 27°C. Olive oil was extracted through centrifugation of each sample (4000 rpm for 5 min). Olive oil samples were stored in dark vials at -20°C.

Determination of oleocanthal and oleacein was done using the kit Aristoleo™ according to the Magiatis-Melliou method which is based on the selective reaction of conjugated aldehydes with reagents of the p-hydroxy-anthranilic acid type to form water-soluble coloured products.

The antioxidant activity was determined spectrophotometrically, by measuring the decrease of the absorbance of DPPH at 517 nm, after adding 6 μl of oil sample into 3 ml of a freshly prepared 100 μM DPPH stock solution in ethyl acetate. Experiments were performed in triplicate. Statistical analysis of the data was done via the use of SPSS.

Results

Measurements were conducted on a total of 32 olive oil samples isolated from three different olive cultivars: Ntopia (12 samples), Thiako (10 samples) and Koroni (10 samples). The 32 samples were similarly distributed in altitude: 13 samples in low altitude (< 150 m), 9 samples in medium altitude (151m - 250 m) and 10 samples in high altitude (>250 m).

Figure 1 shows the geographical distribution of the olive oil samples within the island of Kefalonia by using the following color coding: Red (Ntopia), Green (Thiako) and Blue (Koroni). The mean concentrations of the oleocanthal-oleacein compounds (measured with the kit Aristoleo™) were the following (displayed graphically in Figure 2): Ntopia: 781 mg/Kg (sd: 142 mg/Kg), Thiako: 490 (sd: 165 mg/Kg), Koroni: 250 mg/Kg (sd: 103 mg/Kg).

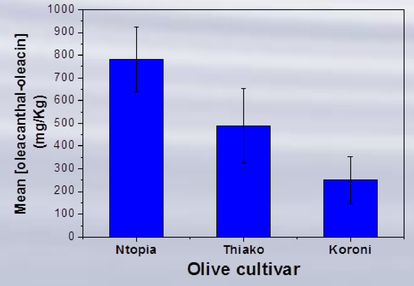

Figure 2. Mean concentrations of the oleocanthal-oleacin compounds

Results (continued)

The ANOVA statistical test was shown to be significant ($F = 40.272$, $p < 0.001$) and the Bonferroni post hoc test showed that these three mean concentrations were statistically different from each other. The same statistical test did not show a statistically significant dependence of the oleocanthal-oleacin concentrations as a function of altitude.

The antioxidant activity of the olive oil samples was expressed in Trolox equivalents (mmol Trolox/l of sample) by using the calibration curve shown in the following figure (Figure 3).

For each sample the DPPH absorption decrease at 517 nm was probed as a function of time for a period of 60 min. In order to determine the total antioxidant activity present in each sample, the time dependent experimental data were fit into an exponential decay curve with a constant factor. It was found that for all samples a bi - exponential decay curve was required of the following type was required in order to provide a satisfactory fit to the data:

$$Y(t) = Y_0 + Y_1*\exp(-t/t_1) + Y_2*\exp(-t/t_2) \qquad \text{(Eq. 1)}$$

In the above Equation 1, Y(t) is the DPPH absorbance at time t, and Y_0, Y_1, Y_2, t_1 and t_2 are fitting parameters.

The constant values Y_0 exported from the fits are then employed in order to determine the total antioxidant activity present in each sample by using the equation of the calibration curve shown in Figure 3.

Figure 4 shows three characteristic decay curves corresponding to each of the three olive cultivars and with the following antioxidant activities: 2.79 mg/Kg (Ntopia), 1,94 mg/Kg (Thiako) and 1.52 mg/Kg (Koroni).

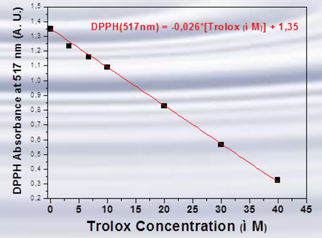

Figure 3. Trolox calibration curve

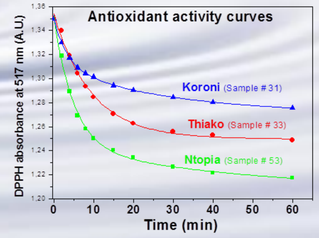

Figure 4. Characteristic DPPH absorbance time- decay curves with superimposed bi-exponential fits

The mean antioxidant activities were the following (displayed graphically in Figure 5):

Ntopia: 2.62 mmol Trolox/l (sd: 0.52), Thiako: 1.86 mmol Trolox/l (sd: 0.51), Koroni: 1.44 mmol Trolox/l (sd: 0.26).

The ANOVA statistical test was shown to be significant ($F = 19.488$, $p < 0.001$) and the Bonferroni post hoc test showed that Ntopia possesses larger antioxidant activity from both Thiako ($p = 0.001$) and Koroni ($p < 0.000$) while the mean values of Thiako and Koroni are statistically similar ($p = 0.147$). The same statistical test did not show a statistically significant dependence of the olive oil antioxidant activity as a function of altitude.

Results (continued)

A relatively large positive linear correlation was observed between the concentration of the oleocanthal-oleacein compounds and the olive oil antioxidant activity ($R^2 \approx 0.70$) as shown in the following figure (Figure 6).

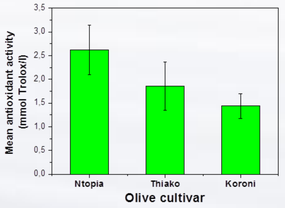

Figure 5. Mean antioxidant activities

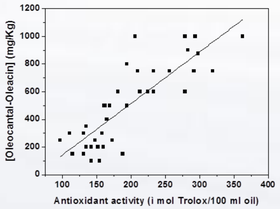

Figure 6. Correlation between antioxidant activity and oleocanthal-oleacin concentrations

Significance

To our knowledge, this is the first study of the nutraceutical aspects of Kefalonian olive oils from three different cultivars.

References

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2. A. Iacono, et al., Effect of oleocanthal and its derivatives on inflammatory response induced by lipopolysaccharide in a murine chondrocyte cell line, Arthritis Rheum. 62 (2010) 1675–1682.

Acknowledgements

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