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# Aromatized olive oils: Influence of flavouring in quality, composition, stability, antioxidants, and antiradical potential

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ABSTRACT

In the present work different flavourings (garlic, hot chili peppers, laurel, oregano and pepper) commonly used in Mediterranean cuisine were added to olive oils from *Cv.* Cobrançosa. Flavouring influence in olive oils quality, fatty acids profile, tocopherols and tocotrienols composition, antiradical activity, total phenols content and oxidative stability were evaluated.

Garlic addition induced an increase in free acidity values (from 0.6 to 0.8%), but the remaining quality indices weren't negatively affected. Fatty acids profile changed but values remained under the limits of extra-virgin olive oils. Olive oils were nutritionally enriched due to the increase in vitamin E, mainly in oils flavoured with hot chili pepper (198.6 mg/kg). Antioxidant properties were influenced as well. Total phenols content decreased in all flavoured olive oils (control with 345.7 mg CAE/kg; oregano 293.8 mg CAE/kg) but the capability to counteract oxidation was generally improved (control with 9.4 h and oregano with 10.4 h). The addition of flavouring influenced quality, composition and olive oils characteristics being possible to separate them according to the flavouring used by applying chemometrics.



## 1. Introduction

According to recent statistics published by the International Olive Council (IOC) the olive oil consumption is increasing in recent years, being predicted to achieve a worldwide consumption level above 3 million tons in 2014 (International Olive Council, 2014). Undoubtedly olive oil sensorial characteristics and health claims are associated with this increase. Besides being a key ingredient of the Mediterranean diet and cuisine, olive oil is related with many health benefits, including the prevention of many modern life-style diseases, like some kinds of cancer (Assmann et al., 1997; Owen et al., 2004) and cardiovascular diseases (Covas, 2007; Fitó et al., 2005).

Consumers are now more informed than ever regarding food products, increasingly demanding for top quality, healthy, and innovative products. In the olive sector, quality products with healthy characteristics have been a constant over the years. Concerning innovation, the recent introduction of flavoured or gourmet olive oils in the market have been the route followed by some industrials. Several kinds of flavourings are used to aromatize olive oils: essential oils (mint and thyme); fruits (apple, banana, bitter-orange and orange, lemon, mandarin); herbs (basil, estragon, fennel, juniper, laurel, lavender, mint, oregano, rosemary, sage, thyme); mushrooms (porcini mushrooms and other truffes); nuts (almonds, hazelnuts, pine nuts); spices (clove, ginger, nutmeg); and vegetables (dried tomatoes, garlic, hot chili peppers, onions, pepper). These flavourings could be added to the olive oil after its extraction, with a defined period of maceration to aromatize the oil, or can be mixed directly with the olive fruits and extracted simultaneously.

The addition of aromatizers to the olive oil influences several characteristics and properties. Their inclusion improves olive oils sensorial characteristics, but the concentration must be kept at low or moderate levels in terms of sensorial acceptability by consumers in order to avoid over-aromatization (Kandylis et al., 2011; Matsakidou, Blekas, & Paraskevopoulou, 2010), particularly for some intense spices (Akçar & Gümüşkesen, 2011; Antoun &

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Tsimidou, 1997; Moldão-Martins et al., 2004). Their quality and shelf-life could be affected as well, since the incorporation of antioxidant and/or pro-oxidant compounds influence olive oils stability. By studying quality indices during storage of flavoured olive oils, Baiano, Terracone, Gambacorta, and La Notte (2009) observed that those with garlic retained their indices below the maximum allowed for extra-virgin olive oils. Gambacorta et al. (2007) reported that the addition of different concentrations of garlic, hot pepper, oregano, and rosemary at long term improved the stability of the olive oils. Some works studied the changes in the oxidative status of flavoured olive oils to verify the efficiency of flavourings bioactive properties and their contribution to olive oils stability. Aromatic plants like rosemary and thyme were capable to protect the oil from thermal oxidation (Ayadi, Grati-Kamoun, & Attia, 2009). Meanwhile lemon and thyme at high concentrations (80 g/kg of oil) weren't efficient to protect the olive oils from thermo-oxidative processes at the smoking point as observed by Issaoui, Flamini, Hajaij, Cioni, and Hammami (2011). The addition of different flavourings is also known to induce the presence and survival of some microorganisms (moulds, yeast and bacteria) according to the concentration and aromatizer used (Ciafardini, Zullo, & Peca, 2004).

With the present work we intend to contribute for the existent knowledge on flavoured olive oils by studying common flavourings in the Mediterranean cuisine (garlic, hot chili pepper, laurel, oregano and pepper). In this sense we studied the effect of those herbs and spices in the quality parameters (free acidity, peroxide value, $K_{232}$, $K_{270}$ and $\Delta K$), fatty acids profile, and tocopherols and tocotrienols content. Total phenols content, antiradical scavenging activity, and oxidative stability were also evaluated to observe the possible role of the flavourings in the bioactive potential and capability to counteract the oxidative reactions in the olive oils.

## 2. Materials and methods

### 2.1. Samples

Monovarietal Cobrançosa extra virgin olive oil from the crop season of 2010/11 was used (composition and properties before spices addition reported in Table 1). The herbs and spices selected were based in the flavourings most commonly used in the Mediterranean cuisine: *Allium sativum* (garlic), *Capsicum frutescens* L. (hot chili pepper), *Laurus nobilis* L (laurel), *Origanum vulgare* L. (oregano), and *Piper nigrum* L. (pepper). All the flavourings were obtained from local markets and were incorporated dried as is in the olive oils (with exception of garlic which was added fresh). After herbs and spices incorporation (10 g/L of olive oil) the olive oils were stored during three months at room temperature (protected from light exposure in static positions) in order to allow a better maceration and extraction of the flavourings into the olive oil. One group was used as control, with no added flavourings. After this storage period the olive oils were dehydrated with anhydrous sodium sulphate, filtered through Whatman no. 4 paper and used for the analytical determinations.

**Table 1**
Quality parameters, sensorial analysis, composition, bioactivity and stability of *Cv.* Cobrançosa olive oil before the addition of different spices.

| | | | |
|---|---|---|---|
| FA (%) | 0.6 ± 0.0 | $C_{16:0}$ | 10.49 ± 0.23 |
| PV (meq. $O_2$/kg) | 2.8 ± 0.3 | $C_{16:1}$ | 0.66 ± 0.03 |
| $K_{232}$ | 2.10 ± 0.08 | $C_{17:0}$ | 0.14 ± 0.02 |
| $K_{270}$ | 0.13 ± 0.00 | $C_{17:1}$ | 0.21 ± 0.02 |
| $\Delta K$ | −0.004 ± 0.001 | $C_{18:0}$ | 2.75 ± 0.06 |
| α-Tocopherol (mg/kg) | 184 ± 0.4 | $C_{18:1}$ | 74.45 ± 0.25 |
| α-Tocotrienol (mg/kg) | n. d. | $C_{18:2}$ | 9.58 ± 0.11 |
| β-Tocopherol (mg/kg) | 0.9 ± 0.1 | $C_{20:0}$ | 0.41 ± 0.03 |
| γ-Tocopherol (mg/kg) | 4.0 ± 0.1 | $C_{20:1} + C_{18:3}$ | 1.04 ± 0.05 |
| Total vitamin E (mg/kg) | 189 ± 0.5 | $C_{22:0}$ | 0.13 ± 0.01 |
| DPPH (μmol/L TE) | 144 ± 8 | SFA | 13.87 ± 0.30 |
| ABTS (μmol/L TE) | 300 ± 4 | MUFA | 75.37 ± 0.20 |
| Total phenols (mg CAE equiv./kg) | 352 ± 18 | PUFA | 10.61 ± 0.06 |
| Oxidative stability (h) | 10.6 ± 0.1 | Sensory analysis | EVOO |

n. d. – not detected; EVOO – extra virgin olive oil according to European Community Regulation EEC/2568/91 and all subsequent amendments.

### 2.2. Quality parameters determination

The quality parameters assessed were free acidity (FA), peroxide value (PV) and specific coefficients of extinction at 232 and 270 nm ($K_{232}$, $K_{270}$, and $\Delta K$). All the mentioned quality parameters were determined according to European Union standard methods (Annexes II and IX in European Community Regulation EEC/2568/91 from 11th July).

### 2.3. Fatty acids composition

Fatty acids were evaluated as their methyl esters after cold alkaline transesterification with methanolic potassium hydroxide solution (Annexes II and IX in European Community Regulation EEC/2568/91 from 11th July) and extraction with n-heptane. The fatty acid profile was determined accordingly to the method described by Malheiro, Casal, Lamas, Bento, and Pereira (2012).

### 2.4. Tocopherols and tocotrienols composition

Tocopherols and tocotrienols composition was determined according to the ISO 9936 (2006), with some modifications as described by Malheiro, Casal, Teixeira, Bento, and Pereira (2013). Tocopherols and tocotrienols standards (α, β, γ and δ) were purchase from Calbiochem (La Jolla, San Diego, CA) and Sigma (Spain), while the internal standard 2-methyl-2-(4,8,12-trimethyltridecyl) chroman-6-ol (tocol) was from Matreya Inc. (Pleasant Gap, PA). Filtered olive oil (50 mg) was mixed with internal standard solution (tocol) and homogenized. The mixture was centrifuged for 5 min at 13,000 rpm and the supernatant obtained analysed by HPLC.

The chromatographic conditions are those reported by Malheiro et al. (2012, 2013). The compounds were identified by chromatographic comparisons with authentic standards, by co-elution and by their UV spectra. Quantification was based on the internal standard method, using the fluorescence signal response.

### 2.5. Radical scavenging activity (RSA)

Olive oil samples with different flavouring were analysed for their antiradical activity by two chemical assays: DPPH (2,2-diphenyl-1-picrylhydrazyl) radical and ABTS (2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonic acid)) radical.

In DPPH assay the method applied was performed accordingly to that described by Kalantzakis, Blekas, Pegklidou, and Boskou (2006) and Malheiro et al. (2012). Briefly, olive oil was diluted in ethyl acetate (100 μL/mL of ethyl acetate) was mixed with a DPPH solution with a concentration of $1 \times 10^{-4}$ mol/L in ethyl acetate. The mixture was then homogenised and kept in the dark for 30 min for reaction. After that the absorbance was registered at $\lambda = 515$ nm against a blank solution.

The ABTS method was applied according to that describe by Sánchez et al. (2007), based on the capacity of a sample to inhibit the $ABTS^{+}$ radical. The $ABTS^{+}$ radical was generated by chemical reaction with potassium persulfate ($K_2S_2O_8$). To 25 mL of ABTS (7 mmol/L) were added 440 μL of $K_2S_2O_8$ (140 mmol/L), being the