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Bioactive substances in black ripe olives produced in Spain and the USA

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ABSTRACT

The aim of this work was to assess the contents of phenolic and triterpenic substances in commercial olives produced in the USA and Spain, which currently use different olive cultivars and elaboration methods The phenolic compounds identified in black ripe olives were hydroxytyrosol, hydroxytyrosol-4-glucoside, hydroxytyrosol acetylated, salidroside, tyrosol, luteolin-7-glucoside, *p*-coumaric acid and comselogoside.The concentration of these substances in American black ripe olives was lower than 50 mg/kg whereas it was higher than 150 mg/kg in Spanish olives regardless of whether the American processors used the Manzanilla cultivar, which contains higher contents of these substances in the harvested fruit than the Hojiblanca cultivar. Moreover, the concentration of triterpenic acids (oleanolic and maslinic) in American black ripe olives was lower than 200 mg/kg whereas it was higher than 400 mg/kg in the Spanish product. These results indicate that the method used by American processors to elaborate black ripe olives gives rise to lower contents in bioactive substances than the Spanish method, and it must be related to the number of alkali/washing cycles used during processing. Hence, olive processors worldwide must keep in mind the increasing consumer demand for healthy products rich in bioactive substances when developing their elaboration methods.

1. Introduction

Among the many different methods existing worldwide to elaborate table olives, the California-style is very popular in the USA where it was created at the end of the XIX century (Luh et al., 2005). Nevertheless, the world production of black ripe olives has increased during recent decades due to their use in pizzas, salads and ready to eat foods. Their shiny black color is very appreciated by consumers in addition to their mild flavor; although consumer preference varies from country to country (Lee et al., 2012; Sansone-Land et al., 2014).

Nowadays, consumers are demanding food products with high quality which are also rich in bioactive substances. Table olives are an important component of the Mediterranean diet with high contents of monounsaturated fatty acids, fiber, minerals and vitamins. They also possess a high concentration in phenolic and triterpenic acid compounds (Romero et al., 2004, 2010).

Olive phenolic compounds, particularly those in olive oil, have attracted the attention of scientists because of their beneficial effects on health such as antioxidant, antitumor, cardio-protective and neuroprotective activities (Hu et al., 2014; Hohmann et al., 2015; Antonini et al., 2015). In addition, the European Food Safety Authority (EFSA) recently approved a health claim stating that the dietary intake of olive oil polyphenols is able to prevent LDL oxidation (EC, 2012), and hydroxytyrosol and its derivatives are the key components to such activity (Caporaso et al., 2015). This is also the major compound in table olives, and the plasma antioxidant status of human increases due to its consumption (Kountouri et al., 2007).

There are numerous factors that can affect the phenolic compound concentration in table olives including the cultivar (Othman et al., 2008; Malheiro et al., 2011; Ramírez et al., 2014), degree of ripening and the method used for processing (Blekas et al., 2002; Romero et al., 2004; Pereira et al., 2006; Boskou et al., 2006; Medina et al., 2013). In particular, the California-style black ripe olive processing leads to a significant reduction in the level of phenolic compounds due to their oxidation during the blackening step (Brenes et al., 1992; Campestre et al., 2002; Melliou et al., 2015).

Triterpenic acids (maslinic and oleanolic acids) are other bioactive substances present in table olives in a very high concentration (Romero et al., 2010; Alexandraki et al., 2014). They also possess beneficial effects on human health such as antitumor and antioxidant activities (Lozano-Mena et al., 2014; Fukumitsu et al., 2015). Similar to phenolic compounds, the concentration of triterpenic acids in table olives depends on the cultivar and method of processing (Romero et al., 2010; Alexandraki et al., 2014). These are lipophilic substances that diffuse into an alkaline medium such as that used for the processing of black ripe olives. Taking into consideration the increasing demand for enriched food in bioactive substances, a study of their content in commercial black ripe olives elaborated in the two main producing

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countries (USA and Spain) was carried out.

It must be noted that the olive cultivar and the method of processing are very different in these countries. Spain is the main producing country of table olives, with the Hojiblanca cultivar being the most commonly employed for making black ripe olives (García et al., 2014). In contrast, the Manzanilla cultivar is the favorite of American processors (Luh et al., 2005). In addition, the length of the American process is greater and the number of alkaline solutions is higher than the Spanish process (Luh et al., 2005; García et al., 2014). Consequently, it is predictable that the concentration of bioactive substances would be lower in the American olives than in the Spanish ones. The aim of this work was to make a survey of the bioactive substances in black ripe olives elaborated in both countries, the USA and Spain.

2. Materials and methods

2.1. Samples

Olives of the Hojiblanca and Manzanilla cultivars were harvested with a green-yellow color in the province of Seville (Spain). They were put into underground tanks containing 10,000 kg of olives and 5000 L of brine acidified with acetic acid and maintained for 9 months under aerobic conditions (De Castro et al., 2007). Five samples of each variety were taken from 5 different tanks for analyses.

To compare the content in bioactive substances of black ripe olives, commercial products were obtained in the USA and Spain. Thirty samples of commercial black ripe olives were purchased in the USA from local markets located in California, Washington DC and Florida. Olives of the Manzanilla cultivar had been elaborated and canned in tins by American processors. Ten of the samples contained large pitted olives and another ten samples medium pitted olives. The rest of the samples had sliced black olives. In addition, thirty samples of black ripe olives of the Hojiblanca cultivar intended for exportation to the USA were obtained from Spanish local processors located in the province of Seville, south of Spain. The product was packed in jars and pouches, and all of the olives were pitted.

All the samples were analyzed in less than 3 months from marketing.

2.2. Analysis of chemical characteristics of olives

The concentration of NaCl was analyzed by titration with a 0.1 N silver nitrate solution, using a potassium chromate solution as indicator. The pH of the pulp and brine was measured with a Metrohm 670 Titro processor (Herisau, Switzerland). The moisture was measured on olive paste by drying in an oven at 105 °C until a constant weight was obtained.

2.3. Analysis of phenolic compounds

Pitted fruits were crushed with an Ultraturrax homogenizer, and 3 g of the paste were mixed with 10 mL of dimethyl sulfoxide (DMSO). After 30 min of resting contact, the mixture was centrifuged at 6000*g* for 5 min, and 0.25 mL of the supernatant were diluted with 0.5 mL of DMSO plus 0.25 mL of 0.2 mM syringic acid in DMSO (internal standard). The mixture was filtered through a 0.22 μm pore size nylon filter, and an aliquot (20 μL) was injected into the chromatograph. A Spherisorb ODS-2 (5 μm, 250 × 4.6 mm, Waters Inc.) column was used. The HPLC system consisted of a Waters 2695 Alliance (Waters Inc., Mildford, MA, USA) with a pump, column heater and auto sampler and the detection was performed with a Waters 996 diode array detector at 280 nm. Separation was achieved using an elution gradient with an initial composition of 90% water (pH adjusted to 2.7 with phosphoric acid) and 10% methanol. The concentration of the latter solvent was increased to 60, 70 and 100% in 5-min periods. The flow rate of 1 mL/min and a temperature of 35 °C were used. Chromatograms were recorded at 280 nm for phenolic compounds (Ramírez et al., 2014).

The identification of the phenolic compounds in the extract was made using a HPLC–MS system that consisted of a Waters 2695 Alliance with a pump, column heater and auto sampler modules, and the detection was carried out with a Waters 2998 photodiode array detector and a mass single-quadrupole detector (QDa, Waters, USA). The QDa mass detector was operated in the negative mode (ESI-), the capillary voltage was set at 0.8 kV, the cone voltage to 15 V, and nitrogen was used as nebulizer gas with the desolvation temperature set at 600 °C. The flow rate was 1 mL/min, and the column, solvent and gradient conditions were the same as mentioned above.

2.4. Analysis of triterpenic acids

These substances were analyzed following the procedure described by Romero et al. (2010) with slight modifications. One gram of dried olive was mixed in a 10 mL centrifuge tube with 4 mL of methanol/ethanol (1:1, v/v) and vortexed for 1 min, centrifuged at 6000*g* for 5 min at 20 °C, and the solvent was separated from the solid phase. This step was repeated six times, and the pooled solvent extract was vacuum evaporated. The residue was dissolved in 4 mL of methanol, which was filtered through a 0.22 μm pore size nylon filter and an aliquot (20 μL) was injected into the liquid chromatograph. The chromatographic system and column were the same as those used for the phenolic compound analysis. The mobile phase (methanol/acidified water with phosphoric acid at pH 2.7, 92:8, v/v) was delivered to the column at a flow rate of 0.8 mL/min and the eluate was monitored at 210 nm. Oleanolic and maslinic acids were quantified using external standards (Sigma, USA).

2.5. Statistical analyses

Statistical comparisons of the mean values for each experiment were performed by one-way analysis of variance (ANOVA), followed by the Duncan's multiple range test ($p < 0.05$) usingSPSS software v. 23.0 (IBM Corp., Armonk, NY).

3. Results and discussion

3.1. Bioactive substances in olives preserved in brine before the darkening process

The phenolic contents of olives of the Manzanilla and Hojiblanca cultivars, which had been preserved for 9 months in acidified brine, are shown in Table 1. A higher concentration of total phenolic compounds was found in olives of the Manzanilla cultivar than Hojiblanca although this trend was not statistically significant due to the great variability detected among samples. Ramírez et al. (2014) also observed these differences in harvested olives of the two cultivars during two consecutive seasons but they again reported great differences among samples. Although there are several factors that can affect the phenolic composition of olives such as area of production and agronomic and climatic conditions, the data reported in the literature indicate that the Manzanilla cultivar and its olive oil possess the highest content in phenolic compounds among olives cultivated worldwide (Romero et al., 2002; Torres and Maestri, 2006; Xiang et al., 2017). In our study, the olives were harvested with the same degree of maturation and processed in the same olive factory.

The main phenolic compounds identified in the pulp of preserved olives were hydroxytyrosol in both cultivars and oleuropein in the Manzanilla cultivar. Hydroxytyrosol-4-glucoside and verbascoside were also detected at a high concentration. It is well-known that oleuropein is the major phenolic compound in harvested olives (Ramírez et al., 2014) but its concentration decreases with time of preservation because of (i) its diffusion to the surrounding brine; (ii) enzymatic hydrolysis; and (iii) chemical hydrolysis. There is a slow diffusion of oleuropein