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Physicochemical composition and antioxidant activity of three Spanish caper (*Capparis spinosa* L.) fruit cultivars in three stages of development

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ABSTRACT

Few studies have been performed on changes to physicochemical and functional properties during caper fruit development. In this work, a comparative study on the evolution of physical, chemical, and nutritive parameters and bioactive compounds of three Spanish caper cultivars was performed for the first time. These fruits were characterized by presenting the exocarp green in all stages of development, with very slight changes, which were not produced by an increase in the synthesis of carotenoids, but by partial degradation of chlorophylls a and b. There was a decrease in the protein content of the caper fruits. The H-TAA was approximately twice as much as the L-TAA. The amounts of total phenols (TPC), total flavonoids (TFC), and total flavonol compounds (TFoC) were very high in all stages of fruit development, as approximately 60% of the total phenols (TPC) were total flavonoids (TFC) and 60% of the TFC were TFoC. Given that the caper is a common perennial xerophytic shrub with a remarkable adaptability to harsh environments, and given the excellent properties of these fruits, it could be considered a species of great interest for its cultivation due to its resilience against climate change.

1. Introduction

The weather is changing due to climate change and global warming. There is already evidence of this process in agriculture, and higher temperatures and more drought mean that agricultural production is being lost (Ray et al., 2015). High temperatures can cause damage to plant tissues as well as their metabolism and physiology, which can cause a decrease in the growth of plants (Ohama et al., 2017), and if the increase in temperature is excessive, it can lead to the death of plants and crop losses (Yamori et al., 2014). Given this situation, xerophilous plants can have great value because they can adapt to global warming. The caper (*Capparis spinosa* L.) is a common perennial xerophytic shrub with a remarkable adaptability to harsh environments (Chedraoui et al., 2017). This species is a plant that grows in dry and arid environments and it is found in Mediterranean regions in both cultivated and non-cultivated wildness. *C. spinosa* can change its leaf, stem, and root structures when adapting to drought conditions (Gan et al., 2013). The leaf, stem, and root of *C. spinosa* under drought conditions were better developed than those under normal conditions (Gan et al., 2013). These characteristics make the caper be considered as a colonizing plant, which in fact, can be found not only cultivated, but also wild among abandoned terraces and growing between rocks. Its distribution stretches from the Atlantic coast of the Canary Islands and Morocco to the Black Sea to the Crimea and Armenia, in the Mediterranean basin and eastward to the Caspian Sea, and into Iran. It grows in North Africa, Europe, West Asia, Afghanistan, and Australia (Inocencio et al., 2006; Fici, 2014). This plant species is of great interest for its medicinal/pharmacological properties and its culinary uses. Its phytochemical importance relies on many bioactive components present in different organs and its cultivation can be of considerable economic value (Chedraoui et al., 2017). Its curative and medicinal properties have been known since ancient times and are linked to the presence of bioactive compounds of an antioxidant nature (flavonoids, flavonols), sugars, alkaloids, vitamins, etc. (Tlili et al., 2011). The plant has been used traditionally to prevent and/or treat a number of health disorders such as diabetes, hepatitis, obesity, and kidney problems (Anwar et al., 2016). Its anticarcinogenic potential (Kulisic-Bilusic et al., 2012), anti-arthritic, anti-inflammatory (Talat et al., 2015), and antibacterial effects (Nabavi et al., 2016) have also been proven. In addition, different parts of capers, like the fruits and caper buds, are usually pickled and added to salads, sauces, and jams (Anwar et al., 2016). Therefore, the caper is not only able to reduce erosion and slow down the desertification process, but can also do so productively, as a cost-effective alternative to other species.

This species has been cultivated since ancient times; however, in recent years its production has decreased significantly worldwide, with

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a decline in caper exports to testimonial values as of 2015 (UN Comtrade, 2017). In Spain, the production of capers buds decreased from 765 tons in 1999 to a production of 61 tons in 2009 (MAPAMA, 2017), mainly due to the cost of labor.

Therefore, the great potential of *C. spinosa* must be valued and its cultivation increased again, especially in areas such as the Mediterranean basin where soils are increasingly more arid, with unique crops of this species or together with other fruit species (Chedraoui et al., 2017), for which it is necessary to thoroughly understand this species. Many investigations have been conducted with caper buds, and several reviews have been carried out on the beneficial properties of this species for health; however, much less attention has been paid to the study of fruits that can be called caper fruits (Arrar et al., 2013), caper berries (Allaith, 2016; Jiménez-López et al., 2018), or caberberry (Legua et al., 2013). Therefore, the objective of this work was to study the physicochemical composition and antioxidant activity of caper fruits. As far as can be ascertained, no comprehensive studies on the development of caper fruits have been done. This is the first paper that studies the evolution of physicochemical parameters, antioxidant activity, and total phenols during three stages of caper fruit development.

2. Materials and methods

2.1. Plant material

Caper fruits of three cultivars, 'Orihuela 4' ('ORI 4'), 'Alberca 2' ('ALB 2'), and 'Alcayna 2' ('ALC 2') were hand-collected during two consecutive seasons, 2016 and 2017. 'ORI 4' was collected in Orihuela (Alicante, Spain), 'ALB 2' in La Alberca (Murcia, Spain) and 'ALC 2' in La Alcayna (Murcia, Spain). All cultivars were grown in rainfed plots and harvested in June, July, and September 2016 and 2017. The caper fruits were classified into three stages of development (Fig. 1): *finos*, with diameters less than 13 mm (thin); *medianos*, between 13–20 mm (medium); and *gruesos*, with diameters larger than 20 mm (thick) (BOE, 1984). These three stages of fruit development correspond to phenological stages 73, 75, and 79, respectively (Legua et al., 2013). One hundred fruits were harvested per stage and cultivar, and after performing non-destructive measures, they were stored the same day at −80 °C.

2.2. Physical parameters

The following physical parameters were measured in 30 fruits per stage and cultivar: equatorial diameter (mm) and fruit length (mm) using a digital caliper (model CD-15 DC; Mitutoyo (UK) Ltd, Telford, UK); fruit weight (g) was measured using a digital balance (model BL-600; Sartorius, Madrid, Spain). Instrumental color was on the surface of the caper fruits at two opposite points of the equatorial zone. Color was assessed according to the *Commission Internationale de l'Eclairage* (CIE*Lab*) and expressed as L^*, a^*, b^*, and Chrome, with a Minolta C-300 Chroma Meter spectrophotometer (Minolta Corp., Osaka, Japan) coupled to a Minolta DP-301 data processor.

2.3. Biochemical parameters

The following parameters were measured in triplicate, with 70 fruits destined to the analysis of biochemical parameters: (i) Chlorophylls a and b were extracted from each sample using 85% acetone (AOAC, 1990). Absorbance was read at 664 and 647 nm, using a Helios Gamma spectrophotometer (model, UVG 1002E; Helios, Cambridge, UK). Results were expressed as mg × 100 g^{-1} fresh weight (fw); (ii) Total carotenoids were extracted according to Valero et al. (2011) with acetone and diethyl ether to promote phase separation. The lipophilic phase was used to estimate the total carotenoid content by reading the absorbance at 450 nm, and the results were expressed as mg of carotenoids × 100 g^{-1} fw, taking into account $\varepsilon_{cm}^{1\%} = 2560$; (iii). The protein content was analyzed by the Bradford (1976) method, using the Bio-Rad reactive and quantified according to Almansa et al. (2016). Results were expressed as mg × g^{-1} fw.

Extracts of caper fruits for the analysis of hydrophilic-total antioxidant activity (H-TAA) and lipophilic-total antioxidant activity (L-TAA) were prepared using Tris-acetate buffer pH 6.0, 20 mM $CaCl_2$, and ethyl acetate to separate the aqueous and organic phases, respectively (Arnao et al., 2001). The results were expressed as mg Trolox equivalent × 100 g^{-1} fw.

For the antioxidant activity determination by the ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl), and FRAP (ferric reducing antioxidant power) methods, a methanol extract was prepared as described by Wojdyło et al. (2013). The free radical scavenging capacities were determined by three methods, ABTS (Re et al., 1999), DPPH· radical (Brand-Williams et al., 1995), and FRAP (Benzie and Strain, 1996). Calibration curves, in the range of 0.5–5.0 mmol Trolox × L^{-1}, were prepared for the three methods and showed good linearity ($R^2 = 0.998$). Antioxidant capacity analyses were run in triplicate, and the results were expressed as mM Trolox fw.

Total phenolic compounds (TFC) were quantified in the hydrophilic phase according to Singleton et al. (1999) using the Folin–Ciocalteu reagent. A calibration curve was performed with gallic acid and the results were expressed as mg GAE x 100 g^{-1} fw.

Total flavonoid (TFC) and total flavonol (TFoC) compounds were extracted following the Zhuang method (1992) with 80% methanol. The analysis of total flavonoids was performed by spectrophotometry following the Zhuang method (1992) with $NaNO_2$, 10% $AlCl_3$, and 1 M

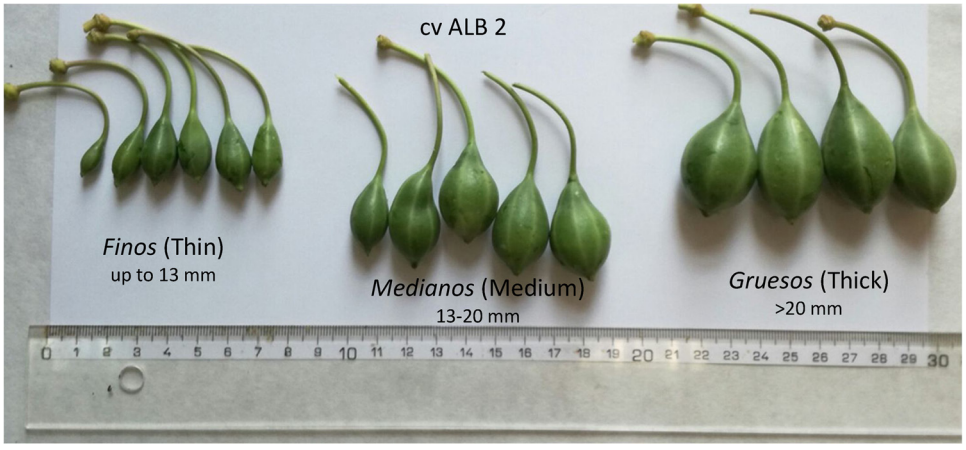

Fig. 1. Different sizes of caper fruits 'ALB 2' cultivar.