Antioxidant Activity and Bioactive Compounds Contents in Different Stages of Flower Bud Development from Three Spanish Caper (Capparis spinosa) Cultivars

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Capparis spinosa L. is cultivated in the Mediterranean basin. In Spain, flower buds, unripe fruits, and tender shoots, either pickled or brined, are used as food. The main objective of the present work was to study the development of total polyphenols, flavonoids, and flavonols contents, total antioxidant activity (TAA) and physicochemical parameters of the flower buds (capers) in six development stages, for the first time, from three cultivars harvested in Spain. Total polyphenols, flavonoids and flavonols contents were very high in all stages of caper development, and were very similar in the three cultivars. The TAA values obtained by the DPPH method, hydrophilic TAA (H-TAA) and total polyphenols, as well as protein and sugar contents, tended to decrease as capers developed and increased in size. Also, H-TAA was significantly higher than lipophilic TAA (L-TAA) in the three cultivars. The results obtained from the current report draw attention to the antioxidant potential of capers, especially those of the ‘Collados 5’ cultivar, and showed that the antioxidant and nutritional properties of capers were better the smaller they were. Increased consumption of capers in the diet and their cultivation should be encouraged.

Key Words: flavonoid, flavonol, polyphenol, pigment, sugar.

Introduction

The caper bush (Capparis spinosa L.) is a common perennial winter-deciduous shrub largely cultivated in the Mediterranean basin. It grows in North Africa, Europe, West Asia, Afghanistan and Australia.

In recent years, interest in consuming foods with health benefits has increased (Wu et al., 2004). Plants have been valued as a rich source of medicinal and nutraceutical compounds for centuries. Among valuable flora, wild plants have gained a lot of attention as a food source and for their potential health benefits. Several members of the Capparis genus, especially Capparis spinosa, have been recognized as a beneficial food because of their high nutritional value and medicinal and pharmacological attributes linked to the presence of antioxidant bioactive compounds (polyphenols, flavonoids), sugars, alkaloids, vitamins, etc. (Anwar et al., 2016). The caper is a xerophytic Mediterranean shrub with a remarkable adaptability to harsh environments. Mediterranean countries are in a region of the world threatened by global warming and C. spinosa is a promising crop for arid or semi-arid regions within the climate change context (Chedraoui et al., 2017), since the caper plant is highly tolerant to drought and heat stress. Therefore, the caper plant’s remarkable ability to adapt to hostile environments, along with its phytochemical importance, suggest that its cultivation could be of considerable economic value.

In Spain, the most valuable parts of Capparis spinosa used as food are the fresh aerial parts, especially the flower buds (capers), unripe fruits and tender shoots. These are pickled or kept in brine and used as an appetizer with olives, cheese, and nuts or as a complement to meat, salads, pasta, and other foods. Spain, Morocco, Italy, Turkey, and Greece are big exporters of caper
buds, while the UK and the USA are big importers (Anwar et al., 2016). Few studies have reviewed *C. spinosa* focusing on the plant’s nutritional qualities, food and medicinal uses, phytochemical properties, biological activities, ethnopharmacology, and crop management (Inocencio et al., 2000). The main objective of this study was to increase knowledge about capers from Spain by analysing the content of total polyphenols, flavonoids and flavonols, and the total (TAA) antioxidant capacities of the caper buds. In addition, the hydrophilic (H-TAA) and lipophilic (L-TAA) antioxidant activities, carotenoids, chlorophylls, proteins, and sugar contents of the unopened flower buds in six development stages of three cultivars (*‘Orihuela 7’, ‘Serón 3’, and ‘Collados 5’*) from the Spanish southeast were also quantified. A better understanding of Spanish cultivars is essential for the selection of high-quality caper genotypes that may be of interest for further nutraceutical studies.

**Materials and Methods**

*Plant materials*

Flower buds (capers) of *C. spinosa* from three cultivars, *‘Orihuela 7’, ‘Serón 3’, and ‘Collados 5’*, were collected from Orihuela (Alicante) (38°5’ N, 0°56’ W, 23.6 m asl), Serón (Almería) (37°21’ N, 2°32’ W, 822 m asl), and Águilas (Murcia) (37°24’ N, 1°34’ W, 21 m asl), respectively. The cultivars were grown in rainfed areas in Spain and hand harvested weekly from May to June in 2016 and 2017 from adult plants. Five plants, similar in vigour and size, were selected from each cultivar.

* Determination of pomological properties

According to the quality standards for foreign trade of capers and caper berries (BOE, 1984), the capers collected were classified by size in six stages of development in the laboratory: Nonpareilles (diameter less than 7 mm); Surfines (between 7–8 mm); Capucines (between 8–9 mm); Capotes (between 9–11 mm); Fines (between 11–13 mm) and Gruesas (over 13 mm) (Fig. 1). These stages of caper development correspond to phenological stage 55 (*Beginning of flower bud swelling*) according to Legua et al. (2013). Equatorial diameter (mm) and length (mm) of capers were measured using a digital caliper with 0.01 mm accuracy, and the length/diameter ratio was calculated. Caper weight (g) was measured using a digital balance with an accuracy of 0.01 g. Three samples of 50 capers were taken per stage and cultivar and after performing non-destructive measures, they were stored the same day at −80°C.

*Total antioxidant activity determination*

Methanolic extracts were individually assessed for their possible antioxidative capacities using three complementary tests. Briefly, 0.1 g of sample was mixed with 10 mL of MeOH/water (80:20, v/v) containing 1% HCl, sonicated at 20°C for 15 minutes and left for 24 hours at 4°C. Then, the extract was again sonicated for 15 minutes and centrifuged at 15,000 g for 10 minutes. A DPPH (2,2-diphenyl-1-picrylhydrazyl) free-radical scavenging assay was carried out according to Brand-Williams et al. (1995). The decrease in absorbance of the mixture was measured spectrophotometrically at 515 nm. An ABTS (2,2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging assay was performed according to method of Re et al. (1999). Plant
extracts were allowed to react with ABTS⁺ solution and the decrease in absorbance was measured at 734 nm. A FRAP (ferric reducing antioxidant power) assay was conducted using the method of Benzie and Strain (1996). The increase in absorbance was measured using a spectrophotometer at 593 nm. Trolox was used as a reference standard and results were expressed as mg Trolox/100 g of fresh weight (FW).

H-TAA and L-TAA were determined by the ABTS⁺ method according to Cano et al. (1998). Briefly, 0.5 g of sample was homogenized with 5 mL of phosphate buffer 50 mM, pH 7.5 and 5 mL of ethyl acetate and centrifuged to 15,000 g for 20 minutes. Both fractions were frozen separately at −80°C. Results were expressed as mg Trolox/100 g FW.

**Determination of total polyphenols, flavonoids and flavonols**

Total polyphenols content was analysed by Folin-Ciocalteu’s phenol reagent method, using gallic acid as the standard according to Singleton et al. (1999). The absorbance was measured at 765 nm and the concentration was calculated as mg of gallic acid equivalents (GAE)/100 g FW.

Methanolic caper extracts were used to estimate the total flavonoids and flavonols using a slightly modified method of Argenti et al. (2012). The extracts were made with 80% methanol and a weight/volume ratio of 1/50 and shaken for 24 hours. Total flavonoids were quantified by a slightly modified method of Chang et al. (2002). The reaction mixture contained 0.5 mL extract, 3.5 mL 95% ethanol, 0.1 mL AlCl₃, 0.2 mL sodium acetate and 2.8 mL distilled water. The reaction was maintained at room temperature for 30 minutes and the absorbance was subsequently measured at 415 nm. Total flavonols were quantified by the method of Kumaran et al. (2007) and the absorbance was measured at 440 nm. The reference standard was rutin and results were expressed as mg of rutin equivalents (RE)/100 g FW.

**Determination of chlorophylls and carotenoids**

Chlorophylls a and b were determined according to Official Methods (AOAC, 1990). Absorbance was read at 645 and 664 nm and the results were expressed as mg/100 g FW. 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 carotenoids content by reading the absorbance at 450 nm, and the results were expressed as mg of β-carotene equivalents/100 g FW, taking into account the ε⁰₉₅ = 2560.

**Determination of proteins and sugars**

The protein content was analyzed by the Bradford (1976) method using the Bio-Rad reagent. A standard curve of pure bovine serum albumin (BSA) was used for quantification according to Almansa et al. (2016). Results were expressed as mg protein·g⁻¹ FW. The sugar profile was quantified according to Hernández et al. (2016), and results were expressed as g/100 g FW.

For all assays, three extracts were prepared by stage, cultivar and year and two determinations were made for each of them (n = 12).

**Statistical analyses**

Statistical analyses were performed using the software package SPSS 18.0 for Windows (SPSS Science, Chicago, IL, USA). A basic descriptive statistical analysis was followed by an analysis of variance test for mean comparisons. The method used to discriminate among the means (Multiple Range Test) was Fisher’s LSD (Least Significant Difference) procedure at a 95% confidence level. Correlations were obtained by Pearson correlation coefficient.

**Results**

**Pomological properties**

The weights of the capers were 0.131–0.79 g, 0.09–0.952 g, and 0.103–0.649 g for ‘Orihuela 7’, ‘Serón 3’, and ‘Collados 5’ cultivars, respectively (Table 1). The caper weights showed significant differences between

| Stage       | Weight (g) | Length/Diameter Ratio |
|-------------|------------|-----------------------|
|             | ORIHUELA 7 | SERÓN 3 | COLLADOS 5 | ORIHUELA 7 | SERÓN 3 | COLLADOS 5 |
| Nonpareilles| 0.131 ± 0.006ₐ | 0.090 ± 0.004ₐ | 0.103 ± 0.005ₐ | 1.064 ± 0.027ₐ | 0.948 ± 0.023ₐ | 0.989 ± 0.023ₐₜₐb |
| Surfines    | 0.170 ± 0.004ₐ | 0.150 ± 0.005ₐ | 0.150 ± 0.004ₐ | 1.048 ± 0.013ₐ | 0.920 ± 0.017ₐ | 0.993 ± 0.011ₐₜb |
| Capucines   | 0.248 ± 0.013ₐ | 0.194 ± 0.007ₐ | 0.219 ± 0.007ₐ | 0.999 ± 0.018ₐ | 0.905 ± 0.013ₐ | 1.004 ± 0.012ₐₜ |
| Capotes     | 0.357 ± 0.012ₐ | 0.328 ± 0.011ₐ | 0.292 ± 0.010ₐ | 0.991 ± 0.011ₐ | 0.879 ± 0.018ₐ | 1.003 ± 0.009ₐₜ |
| Fines       | 0.507 ± 0.015ₐ | 0.465 ± 0.011ₐ | 0.474 ± 0.010ₐ | 0.929 ± 0.023ₐ | 0.929 ± 0.010ₐ | 0.992 ± 0.010ₐₜ |
| Gruesas     | 0.790 ± 0.026ₐ | 0.952 ± 0.054ₐ | 0.649 ± 0.026ₐ | 0.930 ± 0.016ₐ | 0.917 ± 0.09ₐ | 0.956 ± 0.013ₐ \laughter

Values (means ± SE) followed by the same letter were not significantly different according to Fisher’s least significant difference (LSD) test at a 95% confidence level (P<0.05) (n = 150). Different capital letters indicate differences between developmental stages and different lowercase letters indicate differences between cultivars.
the six stages of development, mainly in the Capucines stage; capers of ‘Orihuela 7’ were the heaviest, except for those in the Gruesas stage from the ‘Serón 3’ cultivar, which were significantly heavier.

On the other hand, ‘Orihuela 7’ capers had a length/diameter ratio that decreased as the capers grew (Table 1), going from being more elongated to more rounded. Capers of ‘Collados 5’ showed a length/diameter ratio very close to one in all development stages, and were more rounded from the beginning. Capers of ‘Serón 3’ had a lower ratio in all the stages.

Total antioxidant activity

The TAA of capers was evaluated using three different analytical methods: ABTS•+, DPPH and FRAP (Table 2). TAA by the ABTS•+ method ranged from 192.7–95.1 mg Trolox/100 g FW for the ‘Collados 5’ cultivar and showed only significant differences with ‘Serón 3’ cultivar. The values obtained by the DPPH method were higher (433–1542.5 mg Trolox/100 g FW), and the only significant differences were between cultivars in the Nonpareilles and Capucines stages. TAA tended to decrease as the capers developed in the three cultivars.

In addition, the H-TAA was between 8–24, 12–38 and 32–68 times greater than the L-TAA in the different stages of development in the ‘Orihuela 7’, ‘Serón 3’, and ‘Collados 5’ cultivars, respectively (Fig. 2). The H-TAA was significantly higher in ‘Collados 5’ than in ‘Serón 3’ and ‘Orihuela 7’, but the L-TAA did not show any significant differences between cultivars in any de-

Table 2. Antioxidant activity in the different development stages of ‘Orihuela 7’, ‘Serón 3’, and ‘Collados 5’ caper cultivars.

| Stage      | ORIHUELA 7 | SERÓN 3 | COLLADOS 5 | ORIHUELA 7 | SERÓN 3 | COLLADOS 5 | ORIHUELA 7 | SERÓN 3 | COLLADOS 5 |
|------------|------------|----------|------------|------------|----------|------------|------------|----------|------------|
| ABTS•+     | 132.7±19.3| 80.1±0.5 | 192.7±0.8  | 132.7±39.5 | 45.1±17.0 | 122.6±28.3 | 805.9±11.3 | 943.6±39.3 | 1542.5±180.4 |
| FRAP       | 102.6±5.3  | 82.0±0.5 | 111.5±14.0 | 112.6±20.8 | 120.1±4.2 | 563.3±60.0 | 433.0±73.3 | 562.4±35.8 | 668.8±43.0 |
| DPPH       | 97.6±14.0  | 75.1±29.8 | 112.6±5.5  | 130.2±2.5  | 92.6±6.8  | 433.0±73.3 | 562.4±35.8 | 668.8±43.0 | 681.5±99.1 |

* Values (means±SE) followed by the same letter were not significantly different according to Fisher’s least significant difference (LSD) test at a 95% confidence level (*P* < 0.05) (n = 12). Different capital letters indicate differences between developmental stages and different lowercase letters indicate differences between cultivars.

* Abbreviations: ABTS•+, 2,2’-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical; FRAP, ferric reducing antioxidant power; DPPH, 2,2-diphenyl-1-picrylhydrazyl.

Fig. 2. H-TAA (A) and L-TAA (B) in the different development stages of ‘Orihuela 7’, ‘Serón 3’, and ‘Collados 5’ caper cultivars. Different capital letters indicate significant differences between stages and different lowercase letters between cultivars (*P* < 0.05) (n = 12). Error bars represent the SE of the mean. Abbreviations: H-TAA, hydrophilic total antioxidant activity; L-TAA, lipophilic total antioxidant activity.
development stage. H-TAA decreased slightly as the size of capers increased, while the L-TAA increased slightly, in the ‘Orihuela 7’ and ‘Serón 3’ cultivars.

**Total polyphenols, flavonoids and flavonols content**

The total polyphenols content ranged from 675.5–849.4 mg GAE/100 g FW (Nonpareilles) to 458.1–525.8 mg GAE/100 g FW (Gruesas), indicating the content of polyphenols decreased as the capers developed (Table 3). The content of flavonols was 68–95% of the content of flavonoids in the Nonpareilles stage and increased to values of 83–96% in capers in the Gruesas stage. On the other hand, the flavonoid and flavanol contents were significantly higher in the Nonpareilles stage of ‘Collados 5’ and the Capotes stage of ‘Serón 3’ cultivars.

**Chlorophylls and carotenoids content**

The carotenoid content (Table 4) ranged between 1.3–3.3 mg/100 g FW and was very similar in the three cultivars, except for the Nonpareilles and Surfines stages in ‘Orihuela 7’ that had significantly higher values. In addition, the level of carotenoids did not change during the development of the capers, except in the Gruesas stage of ‘Orihuela 7’ in which it decreased.

The total chlorophylls content (Table 4) ranged between 7.3–21.2 mg/100 g FW, but it was higher in most of the stages of ‘Collados 5’, except for the Nonpareilles and Gruesas stages of ‘Orihuela 7’ and ‘Serón 3’, respectively. The chlorophyll a content was higher than chlorophyll b in the three cultivars (data not shown), and the chlorophyll a/chlorophyll b ratio ranged from 1–2.2 in ‘Orihuela 7’, 1–1.7 in ‘Serón 3’, and 1–2.7 in ‘Collados 5’ cultivars. There was no clear trend between the development stages regarding the chlorophyll content, but there were significant differences between them.

**Pearson’s correlation coefficients**

Correlations between antioxidant activity, total polyphenols, flavonoids, flavonols and carotenoids in the three cultivars are shown in Table 5. In the ‘Collados 5’ cultivar, total polyphenols correlated positively with ABTS⁺⁺, FRAP, and DPPH antioxidant activities. However, flavonoids and flavonols were correlated with ABTS⁺⁺ and DPPH activities. In the ‘Orihuela 7’ cultivar, total polyphenols correlated with ABTS⁺⁺, FRAP, and DPPH activities and flavonoids with ABTS⁺⁺ and DPPH activities. On the other hand, in ‘Serón 3’, total polyphenols correlated with ABTS⁺⁺ and DPPH activities, while flavonoids only were correlated with ABTS⁺⁺.

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**Table 3.** Total polyphenols, flavonoids and flavonols contents in the different development stages of ‘Orihuela 7’, ‘Serón 3’, and ‘Collados 5’ caper cultivars.

| Stage        | TOTAL POLYPHENOLS (mg GAE/100 g FW) | FLAVONOIDs (mg RE/100 g FW) | FLAVONOLS (mg RE/100 g FW) |
|--------------|-------------------------------------|-----------------------------|-----------------------------|
|              | ORIHUELA 7 | SERÓN 3 | COLLADOS 5 | ORIHUELA 7 | SERÓN 3 | COLLADOS 5 | ORIHUELA 7 | SERÓN 3 | COLLADOS 5 |
| Nonpareilles |          |        |          |              |        |          |              |        |          |
| Surfines     | 675.5±92.8 | 719.2±10.3 | 849.4±78.6 | 466.0±15.8 | 440.3±8.8 | 729.5±0.9 | 316.2±11.6 | 342.4±22.3 | 691.7±6.7 |
| Surfines     | 564.4±29.2 | 566.5±103 | 652.3±46.3 | 447.5±37.6 | 557.8±18.9 | 490.6±2.2 | 400.7±17.9 | 534.8±1.6 | 439.7±3.1 |
| Capucines    | 552.2±4.8   | 699±87.6 | 811.4±81.8 | 415.8±10.1 | 531.5±5.7 | 490.5±30.4 | 368.8±13.0 | 465.6±26.4 | 430.6±5.9 |
| Capotes      | 555.7±16.6  | 739.0±43.1 | 757.7±94.6 | 386.9±12.0 | 566.5±11.1 | 482.1±8.7 | 347.2±11.9 | 511.3±13.8 | 431.4±14.0 |
| Fines        | 508.5±72.8  | 532.1±18.9 | 644.6±64.2 | 374.5±3.6 | 526.6±14.2 | 571.3±3.2 | 334.3±8.7 | 477.6±23.8 | 462.1±1.6 |
| Gruesas      | 458.1±6.1   | 476.6±33.4 | 525.8±71.2 | 334.8±40.7 | 442.0±25.5 | 478.6±59.1 | 283.4±65.6 | 424.6±12.4 | 397.5±43.3 |

a Values (means±SE) followed by the same letter were not significantly different according to Fisher’s least significant difference (LSD) test at a 95% confidence level (P<0.05) (n=12). Different capital letters indicate differences between developmental stages and different lowercase letters indicate differences between cultivars.

**Table 4.** Carotenoids and total chlorophylls contents in the different development stages of ‘Orihuela 7’, ‘Serón 3’, and ‘Collados 5’ caper cultivars.

| Stage        | CAROTENOIDs (mg/100 g FW) | TOTAL CHLOROPHYLLS (mg/100 g FW) |
|--------------|---------------------------|----------------------------------|
|              | ORIHUELA 7 | SERÓN 3 | COLLADOS 5 | ORIHUELA 7 | SERÓN 3 | COLLADOS 5 |
| Nonpareilles | 3.0±0.16 | 1.8±0.34 | 2.1±0.05 | 15.9±0.07 | 12.1±0.05 | 14.9±0.04 |
| Surfines     | 3.3±0.44 | 1.8±0.07 | 1.3±0.02 | 13.2±0.11 | 7.3±0.19 | 14.0±0.003 |
| Capucines    | 3.1±0.45 | 2.0±0.04 | 1.7±0.94 | 7.3±0.19 | 11.3±0.18 | 16.2±0.12 |
| Capotes      | 2.8±0.20 | 2.3±0.25 | 2.3±0.46 | 10.9±0.09 | 10.8±0.11 | 16.2±0.12 |
| Fines        | 2.5±0.15 | 1.9±0.44 | 2.8±0.83 | 9.8±0.10 | 8.9±0.03 | 21.2±0.11 |
| Gruesas      | 1.7±0.28 | 2.0±0.08 | 1.3±0.29 | 8.9±0.09 | 17.8±0.1 | 14.9±0.06 |

a Values (means±SE) followed by the same letter were not significantly different according to Fisher’s least significant difference (LSD) test at a 95% confidence level (P<0.05) (n=12). Different capital letters indicate differences between developmental stages and different lowercase letters indicate differences between cultivars.
activity. Total polyphenols, flavonoids, and flavonols are important bioactive compounds that in general contribute to hydrophilic antioxidant activity (H-TAA). These compounds were positively correlated with H-TAA in the ‘Collados 5’ cultivar. However, flavonoids and flavonols were negatively correlated in the ‘Serón 3’ cultivar. Finally, only a negative correlation was found between L-TAA and carotenoids content in ‘Orihuela 7’.

**Protein and sugar content**

The protein content was very similar in most development stages of ‘Serón 3’ and ‘Collados 5’ cultivars (Fig. 3), and in both cases was much higher than the content of the ‘Orihuela 7’ cultivar that had contents between 3.5 and 5 times lower. In the three cultivars, the protein content showed a tendency to decrease as the capers developed.

The total sugar content (Table 6) was much higher in

### Table 5. Pearson’s correlations coefficients for assays of ‘Orihuela 7’ (a), ‘Serón 3’ (b), and ‘Collados 5’ (c) caper cultivars’ constituents.

| Assay or Constituent | Total Polyphenols (mg GAE/100 g FW) | Flavonoids (mg RE/100 g FW) | Flavonols (mg RE/100 g FW) | Carotenoids (mg/100 g FW) |
|----------------------|-------------------------------------|-----------------------------|-----------------------------|---------------------------|
| ABTS (mg Trolox/100 g FW) | \( ^a 0.980*** \) | \( ^a 0.910** \) | \( ^a 0.319 \) |
|                        | \( ^b 0.717 \) | \( ^b 0.755* \) | \( ^b 0.459 \) |
|                        | \( ^c 0.865** \) | \( ^c 0.787* \) | \( ^c 0.896** \) |
| FRAP (mg Trolox/100 g FW) | \( ^a 0.734* \) | \( ^a 0.520 \) | \( ^a 0.109 \) |
|                        | \( ^b -0.222 \) | \( ^b 0.591 \) | \( ^b 0.582 \) |
|                        | \( ^c 0.816** \) | \( ^c 0.023 \) | \( ^c 0.214 \) |
| DPPH (mg Trolox/100 g FW) | \( ^a 0.767* \) | \( ^a 0.809* \) | \( ^a 0.337 \) |
|                        | \( ^b 0.728 \) | \( ^b -0.172 \) | \( ^b -0.489 \) |
|                        | \( ^c 0.671 \) | \( ^c 0.926*** \) | \( ^c 0.979*** \) |
| H-TAA (mg Trolox/100 g FW) | \( ^a 0.578 \) | \( ^a 0.574 \) | \( ^a 0.612 \) |
|                        | \( ^b 0.299 \) | \( ^b -0.607 \) | \( ^b -0.756* \) |
|                        | \( ^c 0.742* \) | \( ^c 0.813** \) | \( ^c 0.926*** \) |
| L-TAA (mg Trolox/100 g FW) | \( ^a -0.795* \) | \( ^a 0.306 \) | \( ^a 0.430 \) |

* Asterisks indicate significant differences: \( * P<0.1, ** P<0.05, *** P<0.01 \), without \( * P \geq 0.1 \).

Abbreviations: ABTS, 2,2’-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid); FRAP, ferric reducing antioxidant power; DPPH, radical 2,2-diphenyl-1-picrylhydrazyl; GAE, gallic acid equivalents; RE, rutin equivalents; H-TAA, total antioxidant activity of the hydrophilic fraction; L-TAA: total antioxidant activity of the lipophilic fraction.

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**Fig. 3.** Proteins in the different development stages of ‘Orihuela 7’, ‘Serón 3’, and ‘Collados 5’ caper cultivars. Different capital letters indicate significant differences between stages and different lowercase letters between cultivars \( (P<0.05) \) \( (n=12) \). Error bars represent the SE of the mean.
‘Collados 5’ and ‘Serón 3’ compared to the ‘Orihuela 7’ cultivar, and was between 1.3 and 6 times higher, depending on the stage of development. The glucose content was higher than that of fructose in ‘Orihuela 7’ and did not change during caper development. However, the glucose and fructose contents were similar in ‘Serón 3’ and ‘Collados 5’ and both decreased during caper development.

**Discussion**

To date, no studies have been published on the changes in weight or the ratio length/diameter of capers between stages of development and different cultivars. ‘Orihuela 7’ capers was significantly heavier than those of ‘Serón 3’ and ‘Collados 5’ cultivars in most development stages. The initial shape of ‘Orihuela 7’ capers changed throughout development as they went from being the most elongated to rounded, and this shape matched in the Gruesas stage in the three cultivar.

These differences could be due to genetic or geographical variations. The quantification of the antioxidant activity in biological samples depends to a great extent on the method used. Three methods were used to evaluate the antioxidant abilities of capers of *Capparis spinosa* cultivars. Bouriche et al. (2011) found that a methanolic extract of *Capparis spinosa* buds from Algeria exhibited a strong scavenging activity against DPPH radicals (53 μg·mL⁻¹), while a methanolic extract of *C. spinosa* fresh fruit averaged 9.06, 6.13, and 8.13 mmol Trolox kg⁻¹ FW by the FRAP, DPPH and ABTS⁺⁺ methods, respectively (Allaith, 2016). These latter values were higher than those we found by the FRAP (45.1–242.8 mg Trolox/100 g FW) and ABTS⁺⁺ (67.6–192.7 mg Trolox/100 g FW) methods, but much lower than those determined by the DPPH method (433–1542.5 mg Trolox/100 g FW). Germano et al. (2002) reported that a methanolic extract of capers showed strong activities in a DPPH assay (EC₉₀: 177.45 μg·mL⁻¹). The differences observed between these values can be explained, at least in part, by the use of different solvents, extraction methods employed and different caper cultivars.

The hydrophilic fractions of the three caper cultivars exhibited a higher antioxidant activity, between 89–443 mg Trolox/100 g FW, in line with the results for caper fruits of Bahrain (Allaith, 2016). The higher antioxidant activity found in the hydrophilic fraction in caper fruits by FRAP (12.8 mmol·kg⁻¹ FW), ABTS⁺⁺ (9.8 mmol·kg⁻¹ FW) and DPPH (6.4 mmol·kg⁻¹ FW) assays indicated the major contributors to the antioxidant activity of caper fruits were water soluble constituents or polyphenol compounds (Allaith, 2016), and could also be the case in capers. H-TAA decreased slightly as the size of the capers increased for all three cultivars.

Capers are rich in polyphenolic compounds and flavonoids that are usually associated with tolerance to high temperatures (Chedraoui et al., 2017). The concentrations of polyphenols and flavonoids vary depending on the extraction method, genetic factors, and climatic/growing conditions of different sites (Tagnaout et al., 2016). Arrar et al. (2013) determined polyphenols and flavonoid contents of 33.5 mg·g⁻¹ DW and 15.8 mg·g⁻¹ DW in *C. spinosa* flowers from Algeria; these values were very similar to those found in caper buds. Inocencio et al. (2000) observed a wide variation in the flavonoid contents of capers from different regions and they proposed that environmental and physiological factors could have important effects. The total flavonoid contents averaged 6.55 mg·g⁻¹ FW, similar to the value obtained for the cultivars in the present study. Maldini et al. (2016) found that the levels of polyphenols and flavonoids in capers of wild and cultivated *C. spinosa* plants collected from different areas of Sardinia (Italy) ranged from 98–149 mg/100 g FW and 82–117 mg/100 g FW, respectively, levels that were five times lower than those reported in this study, indicating that those levels could be different in cultivated and wild capers. However, Tili et al. (2010) showed that leaves and flower buds of *C. spinosa* from different locations in Tunisia were very rich in total polyphenols, containing an average of 3643 and

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**Table 6.** Totals sugars, glucose, and fructose contents in the different development stages of ‘Orihuela 7’, ‘Serón 3’, and ‘Collados 5’ caper cultivars.

| Stage    | Totals sugars (g/100 g FW) | Glucose (g/100 g FW) | Fructose (g/100 g FW) |
|----------|----------------------------|----------------------|-----------------------|
| Nonpareilles | 3.2±0.32, 16.1±0.06 | 8.3±0.25, 11.8±0.22 | 0.3±0.11, 7.8±0.19 |
| Surfinas  | 3.6±0.39, 8.6±1.08 | 4.1±0.37, 4.9±0.07 | 0.8±0.12, 4.5±1.45 |
| Capucines | 3.6±0.92, 6.3±0.48 | 2.8±0.66, 3.0±0.003 | 0.9±0.28, 1.7±0.46 |
| Capotes   | 4.0±0.32, 6.9±0.94 | 2.7±0.05, 2.4±0.37 | 1.3±0.27, 2.2±0.30 |
| Fines     | 4.6±0.48, 4.3±0.04 | 3.2±0.49, 4.1±0.18 | 1.4±0.04, 3.1±0.22 |
| Gruesas   | 2.0±0.04, 5.1±0.41 | 1.1±0.08, 2.2±0.32 | 0.8±0.10, 2.9±0.41 |

* Values (means ± SE) followed by the same letter were not significantly different according to Fisher’s least significant difference (LSD) test at a 95% confidence level (*P* < 0.05) (n = 12). Different capital letters indicate differences between developmental stages and different lowercase letters indicate differences between cultivars.
mental stresses. In our cultivars, the flavonols content for the edible part of onion (39–42 mg/100 g FW), a and analgesics) and they are used as food supplements on account of their high antioxidant activity (Tagnaout et al., 2016). Germano et al. (2002) attributed the antioxidant power of the methanolic extract of Capparis spinosa buds to the presence of the flavonol rutin. According to Tagnaout et al. (2016) this flavonol is synthesized by plants as an adaptation to arid and semi-arid climates and to give protection against various environmental stresses. In our cultivars, the flavonols content was 68–95% of the flavonoids content in the Nonpareilles stage, and it increased to values of 83–96% in capers in the Gruesas stage, suggesting that capers could be a very rich source of flavonols. Comparing the flavonols contents in the Capotes stage (347.2, 511.3, and 431.4 mg RE/100 g FW) with those reported for the edible part of onion (39–42 mg/100 g FW), a food rich in flavonols (Hendler and Rorvik, 2008), we found that capers are a superior source of flavonols.

In the literature, data on C. spinosa carotenoids are limited. In capers in different Tunisian regions, the total carotenoids content ranged between 411.3–3452.5 μg·g⁻¹ FW (Tili et al., 2009), much higher values than those found in capers from Spain. Later, Tili et al. (2010) reported that the content of these compounds in flower buds ranged between 1.14–9.09 mg/100 g FW, values very similar to those in this study (1.3–3.3 mg/100 g FW). However, Özcan and Akgül (1998) reported that the total carotenoids in buds of C. spinosa were between 5.61–17.07 μg·g⁻¹ DW—about 10 times less than in the current study. Ulukapi et al. (2016) found carotenoids content of 21.24 mg·kg⁻¹ in capers from Turkey, values very similar to those found in capers from Spain. These differences may be due to cultivar variations, geographic locations, and different harvesting and extraction techniques (Tili et al., 2009, 2010). Carotenoids are involved in photosynthesis (they transfer light energy to chlorophylls) and in photoprotection of plants over-exposed to sunlight. In capers, lutein (64.9%) and β-carotene (24.4%), neoxanthin (6.7%) and violaxanthin (3.9%) were identified by Tili et al. (2009); they could therefore be used to increase the intake of these compounds in food as they play an important role in human nutrition. The consumption of C. spinosa is not associated with any adverse effects according to the published literature, indicating that C. spinosa is safe to consume (Sher and Alyemeni, 2010).

Considering the correlation results (Table 5), a high correlation between total polyphenols and/or flavonoids with ABTS⁺, FRAP and/or DPPH activities in the three cultivars was found. However, Yadav and Malpathak (2016) observed no correlation between the antioxidant activities and total polyphenols, and flavonoids contents of stem and leaves extracts of Capparis moonii from India. Other antioxidants differ to polyphenols and flavonoids, such as volatile oils, carotenoids, alkaloids, steroids, tannins, glycosides, and vitamins may be responsible of the antioxidant activity (Yadav and Malpathak, 2016). It is well known that total polyphenols, flavonoids, and flavanols are important bioactive compounds that in general contribute to hydrophilic antioxidant activity (H-TAA). These compounds were positively correlated with H-TAA in the ‘Collados 5’ cultivar, but flavonoids and flavonols were negatively correlated in ‘Serón 3’. Allaith (2016) observed that in the hydrophilic fractions of caper fruits the polyphenol content was positively correlated with FRAP (0.59), DPPH (0.610), and ABTS⁺ (0.486) activities and flavonoids content (0.714, 0.587, and 0.652, respectively). In this study, L-TAA and carotenoids contents were negatively correlated in the ‘Orihuela 7’ cultivar. Ulukapi et al. (2016) observed that total carotenoids and total polyphenols content were positively correlated (0.983) in Capparis spinosa buds.

The protein content in the capers in this study was much lower than that found by Özcan and Akgül (1998) and Ulukapi et al. (2016). Their values were 33% and 9.45% respectively, but they determined crude proteins by the Kjeldahl method which does not measure the protein content directly, so that a conversion factor is needed to convert the measured nitrogen concentration to a protein concentration. UV-visible spectroscopic techniques are often preferred because they give rapid and reliable measurements, and are sensitive to low protein concentrations, and can detect protein concentrations as low as 0.001% total weight. The ‘Orihuela 7’ cultivar had contents between 3.5–5 times lower than the ‘Serón 3’ and ‘Collados 5’ cultivars, which had protein levels between 4.6–6.6 and 4.8–7 mg·g⁻¹ FW, respectively.

In the literature, data on the sugars content of C. spinosa are very limited. Özcan and Akgül (1998) found the reducing sugar content ranged from 3.84–4.69 g/100 g FW in capers, while Ulukapi et al. (2016) found that the glucose content was 8.1 g·kg⁻¹ FW, fructose was 14.5 g·kg⁻¹ FW and saccharose was 3.6 g·kg⁻¹ FW in capers from Turkey. All these values were much lower than those found in Spanish cultivars that ranged between 2–19 g/100 g FW; however, no saccharose was detected in any sample and the glucose content was higher than that of fructose in ‘Orihuela 7’. However, the glucose and fructose contents were similar in ‘Serón 3’ and ‘Collados 5’. The highest sugar contents observed in ‘Serón 3’ and ‘Collados 5’ could be due to the fact that they were acting like osmolytes to allow water intake by caper plants in soil with lower water availa-
bility or more saline than that of ‘Orihuela 7’, which was located in a more humid orchard soil. The higher protein contents in these cultivars could act as osmoprotectors because salt stress and water deficit lead to the formation of reactive oxygen species and increased antioxidative enzyme activities in plants (Parida and Das, 2005). In addition, the higher content of proteins and total sugars in ‘Sérón 3’ and ‘Collados 5’ suggests that the capers of these two cultivars could be of greater benefit from a nutritional point of view than those of ‘Orihuela 7’.

The differences found between the three cultivars studied here in the different parameters investigated could be due to genetic or environmental differences because they were located in very different places.

In conclusion, no significant differences were found in the total antioxidant activity of capers quantified by the ABTS**, DPPH, and FRAP methods between cultivars, but the ‘Collados 5’ cultivar had higher TAA values, especially by the DPPH method. The H-TAA content was much greater than the L-TAA content in the three cultivars, with H-TAA being between 32 to 68 times higher in ‘Collados 5’, while the L-TAA content was similar in the three cultivars. Total polyphenols, flavonoids, and flavonols contents were very high in all stages of caper development, and were very similar in the three cultivars. The flavonoid and flavonol content were significantly higher in the Nonpareilles stage of development in the ‘Collados 5’ cultivar. The carotenoids content was very similar in the three cultivars and it did not change during caper development; however, for the TAA values obtained by the DPPH method, H-TAA, total polyphenols as well as protein and sugar contents, decreased as the capers developed and increased in size in all three cultivars. The results obtained in the present study draw attention to the antioxidant potential of capers, especially that of the ‘Collados 5’ cultivar, and showed that the smaller the capers were, the better the antioxidant and nutritional properties. An increased intake of capers in the diet, and their cultivation, should be encouraged.

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