Bioactivities, chemical composition and nutritional value of *Cynara cardunculus* L. seeds

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**ARTICLE INFO**

**Keywords:**  
Antimicrobial effects  
Antioxidant activity  
Cardoon  
Cynara cardunculus L.  
Cytotoxicity  
Globe artichoke  
Nutritional value  
Phenolic compounds

**ABSTRACT**

In the present study, the nutritional value, bioactive properties, and chemical composition of various cardoon (*Cynara cardunculus* L.) genotypes cultivated in central Greece were investigated. The results demonstrated that *Cynara* seeds are a good source of fat and protein, while they also contain considerable amounts of K, Mg, and Fe and low amount of Na. Sucrose, oxalic acid, and \(\alpha\)-tocopherol were the only free sugar, organic acid, and tocopherol isomer respectively, found among the studied genotypes. The most abundant fatty acids were linoleic, oleic and palmitic acid, while PUFAs was the most abundant fatty acid class. All the tested seeds contained only two phenolic compounds, namely 5-O-cafeoylquinic acid and 3,5-O-caffeoylquinic acid, while significant antioxidant activities and cytotoxicity against tumor cell lines and antimicrobial effects were also observed. In conclusion, cardoon seed extracts could be exploited in the food and pharmaceutical industries as alternative sources of natural compounds with bioactive properties.

1. Introduction

*Cynara cardunculus* L. is a herbaceous perennial species, widely used throughout the centuries for food and medicinal purposes throughout the Mediterranean basin (Petropoulos, Pereira, Tzortzakis, Barros, & Ferreira, 2018). It is considered a tolerant species that can be suggested for other uses, such as animal fodder and energy production (Mancini et al., 2019), and the food industry (Almeida & Simões, 2018; Llorente, Obregón, Avilés, Caffini, & Vairo-Cavalli, 2014). Besides, plant rhizomes extracts have been also attributed with significant antioxidant and antimicrobial properties that may find uses in therapeutic and pharmaceutical purposes (Falleh et al., 2008).

Although seeds constitute a small portion of total aerial biomass, several studies have reported their important bioactive properties, mostly due to their high effectiveness in scavenging free radicals (Georgieva et al., 2014). Racciuia, Melilli, Argento, and Scalisi (2013) highlighted the potential of using cardoon whole grains or hulls for alternative purposes due to their high content in phenolic compounds, such as chlorogenic acid, caffeic acid and catechin, while Cajarville, González, Repetto, Alvir, and Rodríguez (2000) suggested the use of seeds for feeding ruminants. Falleh et al. (2008), reported that cardoon seeds contain similar amounts of phenolic compounds, tannins and flavonoids compared to the leaves. In another study, it was also suggested that ethanolic extracts of seeds had higher antioxidant activity than leaves (Georgieva et al., 2014). Similar results have been reported by Khaldi, Chaouachi, Ksouri, and Gazzah (2013) who evaluated polyphenol content of various Tunisian populations of cardoon plant organs and suggested methanolic extracts of seeds have a higher phenolic compound content and antioxidant activity than stems. These antioxidant properties of seed extracts were attributed mostly to hydrolysable polyphenols consisting of tannins, phenolic acids and hydroxycinnamic acids (Durazzo et al., 2013). Moreover, apart from bioactive properties, *Cynara* seeds (globe artichoke and cardoon) present an important nutritional value due to the significant amounts of protein and crude fiber (Foti et al., 1999).

Globe artichoke is usually cultivated for its edible heads, while cardoon is considered an important energy crop, which is mostly...
cultivated for its aerial parts (leaves and stems). Moreover, harvesting of artichoke heads ceases at the end of each growing period due to rapid quality degradation of heads meaning that a great number of heads and seeds remain each year in the field unexploited. So far, most of the research studies in the literature focus on evaluating the use of cardoon seeds and seed press cakes for biodiesel and animal feed production, respectively. Therefore, in the present study nutritional value, chemical composition, and antioxidant and antimicrobial properties of Cynara cardunculus seeds were investigated, while the potential of using them for alternative purposes in the food and pharmaceutical industry was also evaluated.

2. Materials and methods

2.1. Plant material

Seeds of Cynara cardunculus L. genotypes were evaluated regarding their nutritional value, chemical composition and bioactivities. In particular, seeds from six globe artichokes [Cynara cardunculus L. ssp. scolymus (L.) Fiori], two wild artichokes [Cynara cardunculus L. subsp. sylvestris (L.) Fiori], and one cultivated cardoon genotype [Cynara cardunculus L. subsp. altalis DC] were collected from mature heads from plants grown from seeds at the experimental field of the University of Thessaly, whereas in the case of local landraces, seeds were collected in situ from the regions where they are cultivated. Samples of seeds were obtained from fifteen fully productive plants (one mature inflorescence per plant; \( n = 15 \)) at the 8th principal growth stage (code stage 89), as has been previously described by Petropoulos et al. (2018). Details about the locations where seeds were collected and caputula description of the studied genotypes AS1-AS3 and AS5-AS9 have been reported in previous publications of the authors (Petropoulos et al., 2017, 2018). In brief, the studied genotypes included: a) Greek globe artichoke cultivar cv. “Argitiki” (sample AS1; collected in situ); b) local landrace of globe artichoke with dark purple round heads (sample AS2; collected in situ); c) commercial globe artichoke cultivar with green round heads (Sample AS3; collected from the experimental field); d) wild globe artichoke ecotype with green small round heads and bracts with big spines (sample AS5; collected from the experimental field); e) commercial globe artichoke cultivar with dark purple oblong heads and bracts with small spines (sample AS6; collected from the experimental field); f) Greek globe artichoke cultivar cv. “Purple of Attika” (sample AS7; collected from the experimental field); g) wild globe artichoke ecotype with green small flat round heads and bracts with small spines (sample AS8; collected from the experimental field), and h) commercial cardoon cultivar cv. Biando Avorio (AS9; collected from the experimental field). Seeds from local landrace “Nemea” (sample AS4), which forms green oblong heads with bracts having big spines were collected in situ from the region of Corinthia Prefecture (latitude 37° 80’ 92”, longitude 22° 65’ 21’). Before analyses, seed samples were prepared according to the procedure described by Petropoulos et al. (2017). Briefly, seeds were collected from mature inflorescences, ground with a hydro-cooled ball mill (M20 Universal Mill, IKA*-Werke GmbH & Co. KG, Germany), lyophilized, closed in sealed plastic bags and stored until further analysis at \(-80^\circ C\).

2.2. Standards and reagents

Acetonitrile (99.9%), n-hexane (95%) and ethyl acetate (99.8%) were High Pressure Liquid Chromatography (HPLC) grade from Fisher Scientific (Lisbon, Portugal). The water was obtained from a purification system, Millipore Direct-Q (TGI Pure Water Systems, Greenville, SC, USA). Fatty acid methyl esters standard mixture (standard 47885-U) were purchased from Sigma-Aldrich (St. Louis, MO, USA), as also were other standards: sugars, tocopherols, organic acids and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). Phenolic compounds were purchased from Extrasynthèse (Genay, France).

Racemic tocol was purchased from Matreya (Pleasant Gap, PA, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). Foetal bovine serum (FBS), L-glutamine, hank’s balanced salt solution (HBSS), trypsin–EDTA (ethylenediaminetetraacetic acid), penicillin/streptomycin solution (100 U/ml and 100 mg/ml, respectively), RPMI-1640 and DMEM media were from Hyclone (Logan, USA). Acetic acid, ellipticine, sulphorhodamine B (SRB), trypan blue, trichloroacetic acid (TCA) and Tris were from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals and solvents were of analytical grade and purchased from common sources.

2.3. Chemical composition analyses

2.3.1. Nutritional value

Nutritional value of seed samples was assessed by determining moisture, protein, fat, carbohydrates, and ash composition, following AOAC procedures (AOAC, 2016). Briefly, the macronutrient content was determined by the macro-Kjeldahl (N \( \times 6.25 \)) method, petroleum ether Soxhlet extraction, and incineration (600 °C), for moisture, protein, and fat, respectively. The carbohydrate amount was determined by difference, and the energetic value was estimated by applying the formula: Energy (kcal/100 g d.w.) = 4 × (g protein/100 g d.w. + g carbohydrate/100 g d.w.) + 9 × (g fat/100 g d.w.). The results were expressed in g/100 g d.w.

2.3.2. Free sugars

Free sugars were analyzed by HPLC coupled to a refractometer detector (RI) (Barros et al., 2013). Seeds were subjected to an extraction procedure in a water bath (80 °C; 30 min) with ethanol (80%; 40 ml), with the addition of melezitose (5 mg/ml) as IS. After centrifugation, the supernatant was filtered, concentrated under reduced pressure (60 °C), and defiant with ethyl ether (10 ml; three times). The extract was then centrifuged and concentrated (40 °C) to be re-dissolved in water (5 ml final volume) and filtered for injection (0.2 µm nylon filters from Whatman) in a HPLC-Ri system. The HPLC consisted of an integrated equipment with a pump (Knauer, Smartline system 1000, Berlin, Germany), degasser (Smartline manager 5000), auto-sampler (AS-2057 Jasco, Easton, MD, USA), and an RI detector (Knauer Smartline 2300). Data was analyzed using Clarity 2.4 Software (DataApex). The chromatographic separation was achieved with a Eurospher 100-5 NH2 column (4.6 × 250 mm, 5 µm, Knauer) operating at 30 °C (7971 R Graceoellen). The mobile phase was acetonitrile/deionized water, 70:30 (v/v) at a flow rate of 1 ml/min. The identification was carried out by comparison with authentic standard retention times, while quantification was achieved using the IS method (DataApex, Podhorská, Czech Republic), by comparing with calibration curves constructed from authentic standards. The results were expressed in g/100 g d.w.

2.3.3. Organic acids

Organic acids were analyzed according to the method of Barros et al. (2013). In brief, seed samples (2 g) were subjected to an extraction with meta-phosphoric acid (25 ml; 25 °C; 150 rpm; 45 min), and filtered through Whatman No. 4 paper and 0.2 µm nylon filters before injection. The analysis was performed using a Shimadzu 20A series UFLC (Shimadzu Corporation, Kyoto, Japan). Separation was achieved on a SphereClone (Phenomenex, Torrance, CA, USA) reverse phase C18 column (5 µm, 250 mm × 4.6 mm i.d.) thermostatted at 35 °C. The elution was performed with sulphuric acid (3.6 mM) using a flow rate of 0.8 ml/min. Detection was carried out in a PDA, using 215 and 245 nm (for ascobic acid) as preferred wavelengths. The organic acids found were quantified by comparison of the area of their peaks recorded at 215 and 245 nm with calibration curves obtained from commercial standards of each compound. The results were expressed in mg per 100 g of dry weight (dw).

For quantification, standard compounds were used to construct
2.3.4. Tocopherols

Tocopherols were analyzed according to the method of Pereira, Barros, Carvalho, and Ferreira (2013). In brief, BHT (10 mg/ml; 100 μl) and internal standard (IS; tocol: 50 μg/ml; 400 μl) solutions, prepared with hexane, were added to seed samples. The extraction procedure consisted of successively adding methanol (4 ml), hexane (4 ml), and saturated NaCl aqueous solution (2 ml), with 1 min vortex mixing between each step. The extract was then centrifuged (4000g; 5 min) and the clear upper layer containing tocopherols was collected to a vial, protected from light exposure. The described extraction procedure was repeated twice, with hexane, and the combined extracts were dried under nitrogen stream. To perform the analysis, the dried extracts were re-dissolved in n-hexane (2 ml), passed through a sodium sulphate anhydrous micro-column, and filtered (0.2 μm nylon filters from Whatman) to a dark injection vial. The results were expressed in mg/100 g d.w.

2.3.5. Mineral content

Mineral composition analysis was performed in forced-air dried (at 72 °C) and ground to powder seeds, after dry ashing and extraction with 1 N HCl. Atomic absorption spectrophotometry (Perkin Elmer 1100B, Waltham, MA) was used for Ca, Mg, Fe, Mn, Zn, and Cu content determination, while flame photometry (Sherwood Model 410, Cambridge, UK) was used for Na and K content determination.

2.3.6. Fatty acids

The fatty acid profile was characterized after a transesterification procedure and according to the method previously described by Barros et al. (2013). The fatty acids obtained by Soxhlet extraction were subjected to a methylation process with methanol/sulphuric acid/toluene (2:1:1 (v/v/v); 5 ml) in a water bath (12 h; 50 °C; 160 rpm). Deionized water (3 ml) and diethyl ether (3 ml) were added to obtain phase separation and recover the FAME phase, respectively. Sodium sulphate anhydrous micro-columns were used to remove the remaining water. Before injection, the samples were filtered (0.2 μm nylon filter from Whatman) and transferred to a vial with Teflon. FAMEs were then identified through comparison of retention time with standards; Clarity 4.0.1.7 Software (DataApex, Podohradská, Czech Republic) was used.

2.3.7. Phenolic compounds

2.3.7.1. Extraction preparation. The extracts were prepared by stirring (150 rpm; 25 °C) the seed samples (1 g) and methanol/water (30 ml; 80:20 v/v) for 1 h. The extracts were filtered through Whatman paper No. 4 and the residue was subjected to an additional extraction at the same conditions. Methanol was removed from the combined extracts under reduced pressure (rotary evaporator Büchi R-210, Flawil, Switzerland), and the aqueous phase was frozen and freeze-dried (FeeZone 4.5, Labconco, Kansas City, MO, USA).

2.3.7.2. Phenolic compounds analysis. To assess the phenolic composition, the different seed extracts were dissolved in methanol/water (80:20 v/v) to a final concentration of 5 mg/ml and the phenolic profile was obtained using a Dionex Ultimate 3000 UPLC (Thermo Scientific, San Jose, CA, USA) system equipped with a diode array detector (using 280 nm as the preferred wavelength) coupled to an electrospray ionization mass detector working in negative mode (Linear Ion Trap LTQ XL mass spectrometer, Thermo Finnigan, San Jose, CA, USA) according to the procedure previously described by Bessada, Barreira, Barros, Ferreira, and Oliveira (2016). Briefly, the chromatographic separation was achieved with a Waters Spherisorb S3 ODS-2 C18 (3 μm, 4.6 mm × 150 mm, Waters, Milford, MA, USA) column thermostatted at 35 °C. The solvents used were: (A) 0.1% formic acid in water, (B) acetonitrile. The elution gradient established was isocratic 15% B (5 min), 15% B−20% B (5 min), 20−25% B (10 min), 25−35% B (10 min), 35−50% B (10 min), and re-equilibration of the column, using a flow rate of 0.5 ml/min. MS detection was carried out using nitrogen as the sheath gas (50 psi), and the system operated with a spray voltage of 5 kV, a source temperature of 325 °C, and a capillary voltage of −20 V. The tube lens offset was kept at a voltage of −66 V. The full scan covered the mass range from m/z 100−1500. The collision energy used was 35 (arbitrary units). Data acquisition was carried out with the Xcalibur® data system (ThermoFinnigan, San Jose, CA, USA).

For phenolic compound identification, the retention time, UV−vis and mass spectrum of a standard compound (5-O-caffeoylquinic acid) were used; in the case where no standard compound was available, a tentative identification was given according to literature data. The quantification was achieved using a standard compound calibration curve (5−100 μg/ml; 5-O-caffeoylquinic acid ≥99% purity, Extrasynthèse, Genay, France), based on the UV signal. The results were expressed in mg/100 g d.w.

2.4. Bioactive properties

2.4.1. Antioxidant activity

The dried extracts obtained in Section 2.3.7 were re-dissolved in methanol/water (80:20 v/v) to a concentration of 2 mg/ml, and successive dilutions were made to perform the antioxidant activity assays. Four in vitro tests were applied: DPPH radical-scavenging activity, reducing power, β-carotene bleaching inhibition, and lipid peroxidation inhibition by thiobarbituric acid reactive substance (TBARS), as previously described by the authors (Petropoulos, Fernandes, Barros, Ferreira, & Ntatsi, 2015).

2.4.2. Cytotoxicity

Cytotoxicity was evaluated through the sulforhodamine B assay, as previously described by Guimarães et al. (2013), in four tumor cell lines, namely cervical carcinoma (HeLa), hepatocellular carcinoma (HepG2), breast carcinoma (MCF-7), and non-small cell lung cancer (NCI-H460), and in a non-tumor liver primary culture (PLP2). For that purpose, the dried extracts described in Section 2.3.7 were dissolved to a final concentration of 8 mg/ml and further diluted to perform the assay.

2.4.3. Antimicrobial activity

For antimicrobial activity evaluation, the extracts described in the previous Section 2.3.7 were re-dissolved in DMSO 5% to a concentration of 30 mg/ml and further diluted.

Antimicrobial activity assays were carried out using Gram-negative bacteria: Escherichia coli (ATCC 35210), Salmonella typhimurium (ATCC 13311), Enterobacter cloacae (ATCC 35030), and Gram-positive bacteria: Staphylococcus aureus (ATCC 6538), Bacillus cereus (clinical isolate), and Listeria monocytogenes (NCTC 7973), following a previously described procedure (Soković, Glamolića, Marin, Brkić, & van Griensven, 2010).

Antifungal activity was assessed against Aspergillus fumigatus (ATCC 1022), Aspergillus ochraceus (ATCC 12066), Aspergillus niger (ATCC 6275), Penicillium funiculosum (ATCC 36839), Penicillium ochrochloron (ATCC 9112), and Penicillium verrucosum var. cyclopium (food isolate), following the procedure previously described by the authors (Soković & Van Griensven, 2006).

2.5. Statistical analysis

For each genotype, three batch samples seed (n = 3) were analyzed and all the assays were carried out in triplicate. Data were analyzed with one-way analysis of variance (ANOVA), while means comparison was performed with the Tukey’s HSD Test (p = 0.05). The statistical package StatGraphics 5.1.plus (Statistical Graphics Corporation) was implemented for all the analyses.
Results regarding the nutritional value of the tested cardoon genotypes are presented in Table 1. Moisture content of seeds ranged between 5.1% (AS9; cultivated cardoon) and 8.0% (AS4; local landrace “Nemea”), with significant differences among the tested genotypes. Seeds contained significant amount of carbohydrates, which consisted of 44.0–52.2 g/100 g d.w., while they were also rich in fats and protein with contents ranging between 17.3 and 23.7 g/100 g d.w. and 25.7–30.4 g/100 g d.w., respectively. The highest energy value was observed for AS9 due to its highest content of fat (23.7 g/100 g d.w.), whereas AS4 had the lowest energy due to high moisture content (8.0%). The results of our study are in the same range in terms of dry matter and crude protein content with the report of Cajarville et al. (2000) who evaluated the potential of using cardoon seeds as feed for ruminants. Similar moisture contents to our study were observed by Maccarone, Fallico, Fanella, Mauromicale, and Raccuia (1999) and Foti et al. (1999) who also observed differences between globe artichoke, and wild and cultivated cardoon genotypes, while Raccuia and Melilli (2007) suggested that harvesting year and growing conditions may also have an effect on moisture and oil content of cardoon seeds. Moreover, Nouraei, Rahimmalek, Saeidi, and Bahreininejad (2016) reported a significant effect of irrigation regime on seed oil content of globe artichoke seeds with decreasing water availability, resulting in lower oil yields. Considering the perennial nature of the species, the age of plants (years after establishment) is also essential for the production of extended roots system that allows for exploitation of deeper water pools in soil.

Free sugar, organic acid and tocopherol content is presented in Table 1. Sucrose was the only detected free sugar with significant differences between the tested genotypes being observed (0.30 and 0.51 g/100 g d.w. for AS5 and AS9, respectively). Moreover, oxalic acid was the only organic acid identified (143–304 mg/100 g d.w. for AS3 and AS1, respectively), whereas traces of malic and fumaric acids were detected. Oxalic acid is considered as an antinutrient factor and its high dietary intake is not recommended on a regular basis due to increased risk for the development of calcium oxalate crystals and kidney stones (Moreau & Savage, 2009); however, the detected amounts in our study were within safe limits and lower than other edible species, which are considered as rich sources of oxalic acid (Petropoulos et al., 2015). Therefore, consumption of cardoon seeds should be considered as safe, especially when accounting for their low intake on a daily basis. The only tocopherol isoform detected was α-tocopherol in amounts that ranged between 1.21 mg/100 g d.w. (AS4) and 4.46 mg/100 g d.w. (AS8). Therefore, the amount of total free sugars, organic acids, and tocopherols correspond to the amounts of the individual compounds (sucrose, oxalic acid, and α-tocopherol, respectively). To the best of our knowledge, the free sugar, organic acid, and tocopherol content in Cynara seeds is reported for the first time. So far, only seed oil composition has been characterized with α-tocopherol being the main tocopherol detected in Cynara cardunculus L. seed oil by Ferreira-Dias, Gominho, Baptista, and Pereira (2018), followed by δ- and γ-tocopherol, whereas Maccarone et al. (1999) detected only α-tocopherol in grain oils from different Cynara spp. Genotypes, including globe artichoke and wild and cultivated cardoon. According to Zonta and Stancher (1983), tocopherols are very labile compounds and susceptible to oxidation during post-harvest treatments, which could explain the lack of various tocopherol isomers in seeds and the differences between the reports in the literature.

Mineral content is presented in Table 2, with significant differences being observed between the tested genotypes. Significant amounts of K, Ca, Mg and Fe were detected in all the tested seeds, while the Na content was considerably low (12–24 mg/100 g d.w.), which is considered a very important feature from a nutritional point of view, especially for people who follow diets low in Na. Similar values for all the determined minerals have been reported in cultivated cardoon seeds from our team for the same plants harvested a year before the plants of the present study (Petropoulos et al., 2018), which indicates that in seeds from well-established and fully productive plants, variations in mineral content are mostly due to growing conditions.
Table 3

Fatty acids composition (%) of the studied Cynara cardunculus L. seeds (mean ± SD).

|       | AS1     | AS2     | AS3     | AS4     | AS5     | AS6     | AS7     | AS8     | AS9     |
|-------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| C14:0 | 0.155 ± 0.005 | 0.156 ± 0.004 | 0.103 ± 0.002 | 0.213 ± 0.008 | 0.169 ± 0.005 | 0.156 ± 0.001 | 0.173 ± 0.004 | 0.187 ± 0.006 | 0.144 ± 0.003 |
| C15:0 | 0.034 ± 0.002 | 0.037 ± 0.003 | 0.035 ± 0.001 | 0.056 ± 0.005 | 0.032 ± 0.002 | 0.032 ± 0.001 | 0.036 ± 0.001 | 0.037 ± 0.001 | 0.027 ± 0.001 |
| C16:0 | 15.01 ± 0.02  | 12.99 ± 0.03  | 14.40 ± 0.05  | 15.66 ± 0.02  | 14.24 ± 0.01  | 11.90 ± 0.05  | 14.74 ± 0.05  | 15.7 ± 0.2    | 11.4 ± 0.1   |
| C16:1 | 0.198 ± 0.005 | 0.276 ± 0.002 | 0.215 ± 0.003 | 0.356 ± 0.002 | 0.168 ± 0.007 | 0.163 ± 0.001 | 0.219 ± 0.007 | 0.292 ± 0.003 | 0.146 ± 0.004 |
| C17:0 | 0.094 ± 0.004 | 0.086 ± 0.004 | 0.098 ± 0.006 | 0.098 ± 0.004 | 0.086 ± 0.004 | 0.086 ± 0.004 | 0.086 ± 0.004 | 0.086 ± 0.004 | 0.087 ± 0.002 |
| C18:0 | 3.73 ± 0.02  | 3.12 ± 0.01  | 3.34 ± 0.01  | 3.48 ± 0.01  | 3.37 ± 0.01  | 3.18 ± 0.01  | 4.34 ± 0.03  | 3.81 ± 0.01  | 2.96 ± 0.01  |
| C18:1n9c | 19.06 ± 0.02 | 18.05 ± 0.03 | 19.35 ± 0.02 | 14.85 ± 0.04 | 15.57 ± 0.01 | 12.81 ± 0.01 | 17.51 ± 0.01 | 13.95 ± 0.02 | 10.91 ± 0.02 |
| C18:2n6c | 60.15 ± 0.01 | 64.03 ± 0.02 | 60.93 ± 0.04 | 62.04 ± 0.01 | 64.49 ± 0.01 | 64.91 ± 0.01 | 64.91 ± 0.01 | 64.91 ± 0.01 | 64.91 ± 0.01 |
| C18:3n6 | 0.037 ± 0.001 | 0.045 ± 0.001 | 0.037 ± 0.001 | 0.037 ± 0.001 | 0.041 ± 0.001 | 0.041 ± 0.001 | 0.037 ± 0.001 | 0.037 ± 0.001 | 0.037 ± 0.001 |
| C18:3n3 | 0.18 ± 0.01  | 0.121 ± 0.003 | 0.194 ± 0.002 | 0.16 ± 0.01  | 0.112 ± 0.002 | 0.117 ± 0.005 | 0.130 ± 0.001 | 0.120 ± 0.001 | 0.120 ± 0.001 |
| C20:0 | 0.38 ± 0.01  | 0.320 ± 0.004 | 0.343 ± 0.001 | 0.440 ± 0.008 | 0.355 ± 0.002 | 0.305 ± 0.002 | 0.416 ± 0.002 | 0.39 ± 0.02   | 0.289 ± 0.004 |
| C20:1 | 0.133 ± 0.002 | 0.125 ± 0.001 | 0.138 ± 0.005 | 0.113 ± 0.004 | 0.119 ± 0.002 | 0.112 ± 0.008 | 0.112 ± 0.008 | 0.112 ± 0.008 | 0.112 ± 0.008 |
| C20:2 | 0.064 ± 0.005 | 0.074 ± 0.004 | 0.064 ± 0.003 | 0.072 ± 0.001 | 0.072 ± 0.004 | 0.079 ± 0.004 | 0.079 ± 0.004 | 0.079 ± 0.004 | 0.079 ± 0.004 |
| C21:0 | 0.014 ± 0.001 | 0.012 ± 0.001 | 0.013 ± 0.001 | 0.015 ± 0.001 | 0.014 ± 0.001 | 0.015 ± 0.001 | 0.015 ± 0.001 | 0.015 ± 0.001 | 0.015 ± 0.001 |
| C22:0 | 0.16 ± 0.01  | 0.150 ± 0.001 | 0.172 ± 0.008 | 0.19 ± 0.01  | 0.16 ± 0.01  | 0.103 ± 0.006 | 0.144 ± 0.001 | 0.16 ± 0.01   | 0.105 ± 0.001 |
| C23:0 | 0.262 ± 0.004 | 0.223 ± 0.001 | 0.170 ± 0.003 | 1.51 ± 0.01  | 0.587 ± 0.001 | 0.290 ± 0.008 | 0.382 ± 0.002 | 0.573 ± 0.006 | 0.314 ± 0.004 |
| C24:0 | 0.35 ± 0.01   | 0.199 ± 0.001 | 0.382 ± 0.004 | 0.66 ± 0.02  | 0.42 ± 0.04  | 0.42 ± 0.04  | 0.42 ± 0.04  | 0.42 ± 0.04   | 0.42 ± 0.04   |

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; n6/n3: ratio of omega-6/omega-3 fatty acids.

In each row, different letters mean significant differences between samples according to Tukey test (p = 0.05).
Moreover, the ratio of oxalic acid (mg/100 g d.w.)/Ca (mg/100 g d.w.) is higher than 2.5 for all the studied genotypes, which indicates that despite its high Ca content Cynara seeds can be considered as poor Ca sources or decalciﬁers (Guil, Torija, Giménez, Rodríguez-García, & Himénez, 1996) and further studies regarding bioavailability and bioaccessibility of Ca after digestion have to be carried out.

Regarding the fatty acid composition of seeds (Table 3), 17 individual fatty acids were detected in all the studied genotypes, while the most abundant ones were linolenic (60.15%–72.77%), oleic (10.91%–19.35%) and palmitic acid (11.4%–15.7%) all accounting for more than 92.05% of total fatty acids. The highest amount of linoleic acid was detected in seeds of the AS9 genotype, while the highest contents of oleic and palmitic acid were recorded in AS3 and AS4 genotypes, respectively. A similar composition of fatty acids for cultivated cardoon seeds has been previously reported by our team, where a significant effect of harvesting year was also observed (Petropoulos et al., 2018). To the best of our knowledge, this is the first report regarding fatty acid composition of globe artichoke seeds, since most of the studies refer to seed oils of Cynara cardunculus L.

Polyunsaturated fatty acids (PUFA) were the most abundant class of fatty acids, followed by saturated (SFA) and monounsaturated fatty acids (MUFA) detected in similar amounts, while the ratio of PUFA/SFA was higher than 0.45 for all the tested seeds, which is an important nutritional parameter of food products (Simopoulos, 1997). Petropoulos et al. (2018) have also reported high ratios of n-6/n-3 fatty acids in cardoon seeds harvested in two consecutive years, although these values were lower than those reported in the present study mostly due to the lower amounts of n-3 fatty acids. Moreover, the very low amounts of n-3 fatty acids and α-linolenic acid in particular, resulted in very high ratios of n-6/n-3 fatty acids. This finding implies that low amounts of seeds have to be consumed on a daily basis or its consumption has to be followed by supplementary sources of n-3 fatty acids in order to avoid high cumulative intake of n-6 fatty acids and its negative health effects (Simopoulos, 2008).

The phenolic composition of the tested seeds is presented in Table 4. Only two individual phenolic compounds were detected in all the studied seed extracts, namely 5-O-cafeoylquinic acid and 3,5-O-cafeoylquinic acid (Supplementary Material Fig. S1).

5-O-Caffeoylquinic acid was positively identiﬁed in comparison with the commercial standard. While 3,5-O-cafeoylquinic acid ([M − H]− at m/z 515) was identiﬁed based on its elution order, fragmentation pattern and relative abundances in comparison with those reported by Clifford, Knight, and Kuhnert (2005).

The amounts of these two phenolic acids ranged between 22.5 and 35.8 mg/100 g d.w. and 197–418 mg/100 g d.w., respectively. The highest amounts of total phenolic compounds were detected in seeds of the AS9 genotype (435 mg/100 g d.w.), mostly due to the highest content in 3,5-O-cafeoylquinic acid (418 mg/100 g d.w.). The same phenolic compounds have been previously reported by Petropoulos et al. (2018) who studied phenolic compound composition of cultivated cardoon plant parts. In contrast, Falleh et al. (2008) detected both polyphenols and flavonoids in cardoon seeds without however identifying individual compounds, while they also suggested that seeds contained similar amounts of polyphenols and flavonoids to leaf extracts. Moreover, Khalidi et al. (2013) detected polyphenols and flavonoids in wild cardoon seeds in amounts that differed depending on growing region. These differences in phenolic compound composition compared to the literature reports could be attributed to extraction methodology (Brás, Guerreiro, Duarte, & Neves, 2015), as well as to genotype and growing conditions (Lombardo et al., 2010).

Antioxidant activity and cytotoxicity of seed extracts are presented in Table 5. Antioxidant activity determined with multiple in vitro assays showed signiﬁcant differences among the tested genotypes. In particular, genotype AS1 showed the best results in the DPPH and β-carotene bleaching inhibition assays, while extracts from AS2 and AS4 seeds had the best antioxidant properties for reducing power and

### Table 4

| Peak Rt (min) | λmax (nm) | Identification | AS1 | AS2 | AS3 | AS4 | AS5 | AS6 | AS7 | AS8 | AS9 |
|-------------|-----------|----------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1           | 7.5       | 326            | 353 | 353 | 353 | 354 | 354 | 354 | 354 | 354 | 354 |
| 2           | 20.9      | 328            | 515 | 515 | 515 | 515 | 515 | 515 | 515 | 515 | 515 |

In each row, different letters mean signiﬁcant differences between samples according to Tukey test (p = 0.05).
concentration corresponding to 50% of antioxidant activity or 0.5 of absorbance in reducing power assay. Trolox (positive control) EC50 values: 41 µg/ml (reducing concentration responsible for 50% inhibition of growth in a primary culture of liver cells-PLP2 or in human tumor cell lines. GI50 values for Ellipticine (positive power), 42 µg/ml (DPPH scavenging activity), 18 µg/ml (samples tested against HepG2 and MCF-7 cell lines, while seed extracts of AS9 were the most cytotoxic to non-tumor cell lines, since so far the antimicrobial properties of Cynara cardunculus L. refer to various plant parts, such as leaves, flowers, rhizomes and heads (Falleh et al., 2008), with significant effects against bacteria, such as Staphylococcus aureus, Methicillin-resistant Staphylococcus aureus, Bacillus cereus, B. subtilis, Pseudomonas aeruginosa, Enterococcus faecalis, and E. coli, and fungi, such as Aspergillus niger, Penicillium funiculosum, and P. verrucosum var. cyclopium, and Aspergillus against P. ochrochrous. 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antioxidant activities and cytotoxicity against tumor cell lines and antimicrobial effects were also observed. In conclusion, cardoon seed extracts showed significant bioactive properties that could be exploited in the food and pharmaceutical industries as alternative sources of antimicrobial agents and natural preservatives, as well as sources of bioactive compounds.

Acknowledgments

The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) and FEDER under Programme PT2020 for financial support to CIMO (UID/AGR/00690/2013), C. Pereira (SFRH/BPD/114753/2016), A. Fernandes (SFRH/BPD/122650/2016), and L. Barros (SFRH/BPD/114753/2016) grants and L. Barros contract. The authors are also grateful to the FEDER-Interreg España-Portugal programme for financial support through the project 0377/Iberphenol_6_E. The authors are grateful to the Ministry of Education, Science and Technological Development of the Republic of Serbia for Grant No. 173032.

Declarations of interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.03.066.

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