



Chemical composition of cardoon (*Cynara cardunculus* L. var. *altilis*) petioles as affected by plant growth stage

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ABSTRACT

In the present study petioles were collected from cultivated cardoon (*Cynara cardunculus* L. var. *altilis*) plants in central Greece at sixteen harvest dates (samples P1-P16) and nine growth stages (principal growth stages (PGS) 1–9). The main objective of this study was to study the influence of petioles' growth cycle on their chemical composition. The lipidic content, fatty acids, free sugars, organic acids, and tocopherol composition were analyzed. A total of 27 fatty acids were identified, being the palmitic acid (C16:0, 12.42–50%) the most abundant, except for samples P4 and P5 where linoleic acid revealed the highest relative abundance (C18:2n6c, 46.5 and 39.05%, respectively). The α -, β -, and γ -tocopherols were the identified isoforms of vitamin E, with sample P1 recording the highest tocopherol content. The detected organic acids were oxalic, quinic, malic, citric, and fumaric acids with the highest content being observed in sample P1. Sucrose was the major sugar in all the analyzed samples, followed by fructose, glucose, trehalose, and raffinose. The obtained results allowed us to characterize the chemical composition of cardoon petioles throughout its growth cycle and, consequently, to valorize the species through the exploitation of underutilized plant tissues.

1. Introduction

Cynara cardunculus L., also commonly known as cardoon, belongs to the Asteraceae, and is a species that presents higher economic interest due to its multifaceted industrial potential (Ferioli & D'Antuono, 2021). This species comprises three varieties. The variable *altilis* DC, also called domesticated cardoon, the variable *sylvestris* (Lamk) Fiori or wild cardoon, and the globe artichoke or variable *scolymus* (L.) Fiori (Conceição et al., 2012; Zayed, Serag, & Farag, 2020).

In recent decades the number of industrial applications of the species has been gradually increasing. Initially used as a coagulation agent in the production of protected designation of origin (PDO) cheeses (Amira, Besbes, Attia, & Blecker, 2017), it is currently also involved in the production of bioenergy, biodiesel, biomethane (Amira et al., 2017; Domínguez, Montiel-Rozas, Madejón, Diaz, & Madejón, 2017; Gominho, Curt, Lourenço, Fernández, & Pereira, 2018), as well as animal forage (Cabiddu et al., 2019). The industrial potential of cardoon and its characteristics of high resistance and productivity in geographical areas

with adverse climatic conditions (e.g. extreme temperatures, high salinity, water scarcity, degraded soils), typical of countries in the Mediterranean basin, contributes to the increase in associated interest in this kind of cheap and accessible maintenance (Mirpoor, Giosafatto, & Porta, 2021; Vergara, Ladero, García-Ochoa, & Villar, 2018). As a result of the diversified industrial potential of cardoon, its cultivation represents an important source of income for countries, such as Italy, Greece, Spain, and southern France (Ferioli & D'Antuono, 2021; Vergara et al., 2018). However, the amounts of bio-waste generated throughout the production-processing chain also increase, resulting in tons of plant material that is not properly used and contributing to the increase in environmental pollution. Annually, approximately 14.6 t/ha of dry biomass of cultivated cardoon (var. *altilis*) and 7.4 t/ha of wild cardoon (var. *sylvestris*) are discarded or used for low added value purposes (e.g. animal forage) (Mirpoor et al., 2021). Therefore, the exploitation and the complete characterization of crop by-products are very important. This knowledge is a significant contribution to the sustainable management of bulky waste and the identification of new plant matrices as

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sources of potent biomolecules (Gominho et al., 2018; Jiménez-Moreno et al., 2019). Cardoon is used since ancient times as food and medicinal plant. It is commonly used in the preparation of soups, salads, and sweets. It is also used in folk medicine due to its properties as an anti-diabetic, choleric, diuretic, cardiotoxic, and anti-hemorrhoidal agent (Gostin & Waisundara, 2019; Ramos et al., 2017; Salem et al., 2017). Recent studies have proven its antioxidant, anti-inflammatory (Mandim et al., 2021a), cytotoxic (do Carmo, Pressete, Marques, Granato, & Azevedo, 2018; Mandim et al., 2021a; Mandim et al., 2021b), and antimicrobial potential (Mandim et al., 2021a; Mandim et al., 2022; Scavo et al., 2019). Along with the bioactive potential demonstrated, several studies refer to the wide variety of bioactive compounds found in the distinct vegetable tissues of cardoon. Particularly mono- and dicaffeoylquinic acids, anthocyanins, and other flavonoids, such as apigenin and luteolin derivatives (Ierna, Sortino, & Mauromicale, 2020; Mandim et al., 2021b; Petropoulos, Fernandes, Tzortzakakis, et al., 2019), as well as fiber, proteins, vitamins, and minerals (Zayed et al., 2020).

Over time, several reports have proven the close relationship between the chemical composition of the species with parameters such as growing conditions, geographic location, plant tissue (Mandim, Dias, et al., 2020; Petropoulos, Fernandes, Tzortzakakis, et al., 2019; Petropoulos, Pereira, Tzortzakakis, Barros, & Ferreira, 2018), maturity stage (Mandim, Petropoulos, Fernandes, et al., 2020; Mandim, Petropoulos, Giannoulis, Dias, et al., 2020), or genetic background (Dias et al., 2018), among others. This variability necessitates that the species must be appropriately characterized and exploited, to obtain the maximum potential and increase the added value of the cardoon crop through the sustainable recovery of waste. In this context, the present study was designed to evaluate the influence of the petiole maturation state on the chemical composition of cardoon harvested in central Greece. Parameters such as lipidic content, fatty acid composition, free sugars, tocopherols, and organic acids from sixteen maturation stages throughout the growing cycle were analyzed. The obtained results can be an important contribution toward the selection of the maturation state that presents the highest amounts of targeted bioactive compounds. And simultaneously to the valorization of all plant tissues of cardoon, to add value and interest to the species, and simultaneously reduce the generated crop by-products.

2. Materials and methods

2.1. Plant material

The petioles of *Cynara cardunculus* L. var *altilis* DC cv. *Bianco Avorio* (Fratelli Ignori Spa, Milano, Italy) were harvested at the experimental farm of the University of Thessaly located in Velesino, Greece (22.756 E, 39.396 N) throughout the growing cycle of the crop (September to August). The petioles were harvested at different principal growth stages (PGS). According to the Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie (BBCH) scale the main stages of petioles maturation are comprised between PGS 1 and 9 (Table 1).

Climatic conditions and sampling were previously described by Mandim et al. (2021b). In brief, samples P1, P2, and P3, all with PGS 1, were collected in September, October, and early November, respectively; sample P4 was collected at the end of November (PGS 2); while samples P5 (PGS 3), P6 (PGS 3/4), P7 (PGS 4), and P8 (PGS 5) were collected at monthly intervals at the beginning of each month from January to April; samples P9 (PGS 5), P10 (PGS 5/6), and P11 (PGS 6) were collected at the end of April, and the beginning and the end of May, respectively; there was an overlapping in PGSs 6–8, therefore samples P12, P13 and P14 which were collected at the beginning of June, and the beginning and the end of July, respectively, were the P12 (PGS 6/7), P13 (PGS 7/8), and P14 (PGS 8); finally, samples P15 (PGS 8/9) and P16 (PGS 9) were collected at the beginning and the end of August, respectively. Fresh tissues of petioles were cleaned with distilled water, dried with soft tissue, and put in storage (−80 °C). Then, all the tissues were

Table 1

Main characteristics of *Cynara cardunculus* L. var. *altilis* petioles harvested at different maturation stages.

Sample code	Harvesting time	Principal growth stage	Main plant characteristics
P1	15 September 2017	PGS 1	First leaves visible (up to 3–4 leaves)
P2	15 October 2017	PGS 1	First leaves visible (up to 3–4 leaves)
P3	15 November 2017	PGS 1	First leaves visible (up to 3–4 leaves)
P4	30 November 2018	PGS 2	Rosette starts to develop (up to nine leaves)
P5	09 January 2018	PGS 3	Ground coverage 10–50%
P6	8 February 2018	PGS 3/4	Ground coverage 50–100%
P7	8 March 2018	PGS 4	Leaves achieve 10–50% of full biomass
P8	7 April 2018	PGS 4/5	Leaves achieve 50–100% of full biomass
P9	26 April 2018	PGS 5	Stem elongation and main inflorescence visible
P10	10 May 2018	PGS 5/6	Higher order inflorescences are developed
P11	24 May 2018	PGS 6	First flowers are visible
P12	12 June 2018	PGS 6/7	50% of heads in full blossom up to the end of flowering
P13	4 July 2018	PGS 7/8	Heads are formed and reach full size
P14	18 July 2018	PGS 8	Primary head starts to ripen
P15	9 August 2018	PGS 8/9	50% of heads are ripen up to senescence
P16	29 August 2018	PGS 9	Full senescence, old plants are dead and new leaves are visible

lyophilized and ground to powder (approx. 20 mesh) with a mortar and pestle. For chemical analyses, all the samples were subjected to extraction protocols as described in the following sections.

2.2. Evaluation of the chemical composition

2.2.1. Fatty acids

For the analysis of the fatty acid composition, the extraction of the lipidic fraction was performed following the procedures recommended by the Association of Official Agricultural Chemists (AOAC, 2019). The lipidic fraction, obtained by Soxhlet extraction with petroleum ether, was subjected to a transesterification procedure and the fatty acid content was analyzed in a gas–liquid chromatography (GC 1000, DANI Instruments, Contone, Switzerland), equipped with a flame ionization detector (FID) and a split/splitless injector. The analytical conditions used have been previously described by Dias et al. (2015). The identification and quantification of the fatty acids were performed with the Clarity DataApex 4.0 software (DataApex, Prague, Czech Republic) and by the comparison of chromatographical profile and retention times of the detected Fatty Acid Methyl Ester (FAME mix) peaks with commercial standards (reference standard mixture 47885-U; Sigma Aldrich, St. Louis, MO; USA). The obtained results were presented as relative percentages of each identified fatty acid.

2.2.2. Tocopherols

For the analysis of the composition of tocopherols in the cardoon petioles, the procedure previously described by Ayache et al. (2021) was followed. The petioles samples (500 mg) were extracted with a solution of butyl-hydroxy-toluene (BHT, 10 mg/mL; 100 µL) and tocol (internal standard (IS), 50 mg/mL; 400 µL). To the obtained solution were consecutively added methanol (4 mL), hexane (4 mL), and saturated NaCl aqueous solution (2 mL), while before the addition of each compound the mixture was homogenized by a vortex. After centrifugation (5 min at 4000 g), two layers were formed, and the upper one was

transferred to a vial containing anhydrous sodium sulfate and finally dried under a nitrogen stream. Finally, the samples were re-dissolved in *n*-hexane (2 mL) and analyzed by high-performance liquid chromatography (HPLC, Knauer, Smartline system 1000, Berlin, Germany), coupled to a fluorescence detector (FP-2020, Jasco, Easton, USA), having been applied an excitation of 290 nm and an emission of 330 nm. The chromatographic conditions used were previously described by Dias et al. (2015). The identification and quantification of tocopherols were achieved with the Clarity 2.4 software (DataApex, Podohradská, Czech Republic), using the internal standard method and by comparison with commercial standards (α , β , γ , and δ isoforms). The results obtained were expressed in μg per 100 g of dry weight.

2.2.3. Free sugars

Petioles samples (1 g) were extracted with a hydroethanolic solution (80:20, v/v; 40 mL) and melezitose (internal standard (IS), 25 mg/mL; 1 mL) in a water bath at 80 °C for 90 min. After filtration, the ethanol was evaporated under reduced pressure, and the mixture was washed with diethyl ether. The obtained solution was filtered and analyzed by High-Performance Liquid Chromatography (HPLC, Knauer Smartline 2300, Knauer, Berlin, Germany), coupled with a refractive index detector (RI detector, Knauer Smartline 2300, Knauer, Berlin, Germany). The chromatographic conditions used were previously described by Pires et al. (2021). The identification and quantification of free sugars were performed using the software Clarity 2.4 (DataApex, Prague, Czech Republic), and by comparison of the detected compounds with available commercial standards (D(-)-fructose, D-(+)-sucrose, D-(+)-glucose, D-(+)-trehalose, and D-(+)-raffinose pentahydrate; all purchased by Sigma-Aldrich, St. Louis, MO, USA). The obtained results were presented in g per 100 g of dry weight.

2.2.4. Organic acids

The analysis of the composition of organic acids in cardoon petioles collected at different maturation stages was carried out according to the procedure previously described by Ayache et al. (2021). The petioles samples (1.5 g) were extracted with a solution of metaphosphoric acid (4.5%, w/v; 25 mL) at room temperature for 20 min, under vigorous agitation, and protection from the light. The solutions obtained were filtered through 0.22 μm nylon filter and analyzed by Ultrafast Liquid Chromatography (UPLC, Shimadzu 20A series, Kyoto, Japan), coupled to a Diode Array Detector (UFLC-PDA, Shimadzu Corporation, Kyoto, Japan). The identification and quantification of organic acids were carried out by comparing the spectra and retention times obtained with the commercial standards (oxalic - $y = 1 \times 10^6x + 231891$, $R^2 = 0.9999$; LOD = 12.55 $\mu\text{g}/\text{mL}$, LOQ = 41.82 $\mu\text{g}/\text{mL}$; citric - $y = 1 \times 10^5x + 10277$, $R^2 = 0.9997$; LOD = 10.47 $\mu\text{g}/\text{mL}$, LOQ = 31.91 $\mu\text{g}/\text{mL}$; quinic - $y = 671557x + 14583$, $R^2 = 0.9998$; LOD = 24.18 $\mu\text{g}/\text{mL}$, LOQ = 80.61 $\mu\text{g}/\text{mL}$; malic - $y = 950041x + 6255.6$, $R^2 = 0.9999$, LOD = 35.76 $\mu\text{g}/\text{mL}$, LOQ = 119.18 $\mu\text{g}/\text{mL}$, and fumaric acids - $y = 1 \times 10^7 + 614399$, $R^2 = 0.9986$; LOD = 0.08 $\mu\text{g}/\text{mL}$, LOQ = 0.26 $\mu\text{g}/\text{mL}$ - purchased from Sigma-Aldrich, St. Louis, MO, USA) and the respective calibration curves. The results obtained were expressed in g per 100 g of dry weight.

2.3. Statistical analysis

The assays described were performed in triplicate and the results obtained were presented as mean values \pm standard deviation. Means and standard deviations were calculated using Microsoft Excel. While the SPSS Statistics software (IBM SPSS Statistics for Mac OS, Version 26.0; IBM Corp., Armonk, NY, USA) was used to assess statistical differences between means. An analysis of variance (ANOVA) was performed, and the existence of significant differences between means was analyzed using Tukey's honest significant test (HSD) ($p = 0.05$).

3. Results and discussion

3.1. Lipid fraction and fatty acid composition

The results obtained regarding the lipidic fraction and fatty acid composition (relative percentage), as well as the distribution of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA), are presented in Table 2. A representative chromatogram of the profile of fatty acids identified in cardoon petioles is shown in Fig. 1. The highest lipidic level was recorded in sample P9 with PGS 5 (10.1 g/100 g dw). Whereas the lowest overall content was observed for sample P5 with PGS 3 (0.74 g/100 g dw). Regarding the individual compounds, a total of 27 fatty acids were identified in the analyzed petioles samples. Palmitic acid (C16:0, 12.42–50%) had the highest relative abundance in practically all samples analyzed, except for samples P4 and P5 for which linoleic acid was the most abundant (C18:2n6c, 46.5% and 39.05%, respectively). In turn, eicosadienoic (C20:2) and eicosapentaenoic (C20:5n3) acids were those that showed the lowest relative contents (0.12–0.32 % and 0.021–14.0 %, respectively). For the rest of the fatty acids, a variable content was recorded among the tested samples. In particular, oleic acid was the most abundant in sample P16 (48.3%), while stearic, linolenic, lignoceric, palmitoleic, myristic, and nervonic acids were the richest in P3 (13.2%), P5 (11.08%), P11 (9.8%), P1 (8.7%), P6 (8.2%), and P6 (7.1%), respectively. Saturated fatty acids (SFA) were the most abundant class of fatty acids in practically all maturation stages analyzed in this work (44.0–81.1%), except for samples P4, P5, and P12, in which polyunsaturated fatty acids (PUFA) showed higher relative abundances (50.8–58.4%), and for sample P16, where the presence of monounsaturated fatty acids (MUFA) stood out (52.3%). The variability in lipidic fraction content of petioles harvested at different growth stages could be attributed to temperature fluctuations since the temperature is the most important environmental factor that affects enzymes expression (fatty acids desaturases) that are involved in the unsaturation of C18 saturated fatty acids (He, Qin, Wang, & Ding, 2020).

The results obtained demonstrate that the state of maturation influences the fatty acid composition, with statistically significant differences ($p < 0.05$) between the analyzed samples. These results are in agreement with some of the previous studies with other plant tissues of cardoon conducted by our team, where the fatty acid composition was also influenced by the maturation of the samples (Mandim, Petropoulos, Fernandes, et al., 2020; Mandim, Petropoulos, Giannoulis, Santos-Buelga, et al., 2020; Mandim et al., 2022). In particular, in all these studies which were carried out under similar growing conditions, considering that the different plant tissues were collected from the same experimental plants, it was found that samples with PGS between 5 and 8 were those with the highest lipidic content, more specifically: heads at PGS 5 (Mandim, Petropoulos, Fernandes, et al., 2020), bracts at PGS 5/6 (Mandim, Petropoulos, Giannoulis, Santos-Buelga, et al., 2020), and seeds at PGS 8 (Mandim et al., 2022). In general, palmitic, oleic, and linoleic acids were the most abundant fatty acids in all plant tissues (Mandim, Petropoulos, Fernandes, et al., 2020; Mandim, Petropoulos, Giannoulis, Santos-Buelga, et al., 2020; Mandim et al., 2022). Although the current study showed that a variety of fatty acids is present in petioles, in other studied tissues a higher number of compounds were identified, e.g., thirty fatty acids were identified in the heads, 29 in the bracts, and 17 in the seeds. The variability observed in fatty acids composition among the tested harvesting stages could be attributed to differences in climate conditions throughout the growing period, as also reported by Curt et al. for cardoon seed oil (Curt, Sánchez, & Fernández, 2002). The significance of growing conditions was also highlighted in the case of safflower seeds, where fatty acid composition and oil content were both affected (Ashrafi & Razmjoo, 2010; Roche, Mouloungui, Cerny, & Merah, 2019; Samancı & Özkaynak, 2003; Zemour et al., 2021). For their part, Abudurehman, Liu, Zhang, & Guan, (2014) suggested that climate change with increasing temperatures may affect

Table 2
Lipidic fraction and fatty acids composition of *Cynara cardunculus* L. var. *atilis* petioles in relation to maturity stage.

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16
Total lipidic fraction (g/100 g dw)	1.2 ± 0.1 _i	4.0 ± 0.4 ^f	1.2 ± 0.1 ⁱ	1.2 ± 0.1 ⁱ	0.74 ± 0.02 ⁱ	3.2 ± 0.2 _{gh}	3.7 ± 0.2 _{fg}	6 ± 1 ^d	10.1 ± 0.2 ^a	6.5 ± 0.3 ^c	2.7 ± 0.2 ^h	7.0 ± 0.1 _{bc}	7.5 ± 0.1 _b	4.2 ± 0.1 ^f	3.1 ± 0.2 _{gh}	4.8 ± 0.4 _e
Fatty acids (relative percentage, %)																
C6:0	2.7 ± 0.1 _a	0.98 ± 0.01 ^d	1.85 ± 0.04 ^b	0.207 ± 0.001 ⁱ	0.34 ± 0.02 ^h	0.55 ± 0.03 ^g	1.1 ± 0.1 ^c	0.80 ± 0.02 ^e	0.60 ± 0.03 ^{fg}	0.98 ± 0.04 ^d	0.24 ± 0.01 ⁱ	0.17 ± 0.01 ⁱ	0.337 ± 0.005 ^h	0.412 ± 0.004 ^h	0.67 ± 0.02 ^f	0.96 ± 0.05 ^d
C8:0	1.09 ± 0.03 ^b	0.323 ± 0.002 ^{fgh}	0.33 ± 0.03 ^{efg}	0.18 ± 0.01 ^k	0.284 ± 0.001 ^{ghi}	1.98 ± 0.01 ^a	0.24 ± 0.02 ^{ij}	0.275 ± 0.001 ^{hi}	0.252 ± 0.003 ⁱ	0.52 ± 0.05 ^d	0.19 ± 0.01 ^{jk}	0.252 ± 0.001 ⁱ	0.38 ± 0.02 ^e	0.9 ± 0.1 ^c	0.18 ± 0.01 ^k	0.36 ± 0.01 ^{ef}
C10:0	1.02 ± 0.04 ^b	0.38 ± 0.02 ^f	0.46 ± 0.02 ^e	0.58 ± 0.03 ^d	0.171 ± 0.001 ^h	1.80 ± 0.01 ^a	0.47 ± 0.04 ^e	0.22 ± 0.01 ^h	0.47 ± 0.03 ^e	0.55 ± 0.01 ^d	0.080 ± 0.001 ⁱ	0.110 ± 0.005 ⁱ	0.39 ± 0.01 ^f	0.88 ± 0.02 ^c	0.30 ± 0.01 ^g	0.183 ± 0.001 ^h
C11:0	1.9 ± 0.1 _a	1.09 ± 0.02 ^{cd}	1.1 ± 0.1 ^{cd}	0.534 ± 0.002 ^g	0.36 ± 0.03 ^h	0.58 ± 0.01 ^{fg}	1.8 ± 0.1 ^b	1.2 ± 0.1 ^c	0.7 ± 0.1 ^f	1.1 ± 0.1 _d	0.84 ± 0.02 ^e	0.58 ± 0.03 ^{fg}	0.36 ± 0.02 ^h	0.87 ± 0.05 ^e	0.22 ± 0.01 ⁱ	0.39 ± 0.02 ^h
C12:0	0.54 ± 0.04 ^h	0.74 ± 0.05 ^g	0.26 ± 0.03 ⁱ	0.23 ± 0.01 ⁱ	0.3 ± 0.1 ⁱ	23.56 ± 0.01 ^a	0.47 ± 0.01 ^h	2.78 ± 0.03 ^d	2.0 ± 0.1 _e	3.91 ± 0.02 ^c	2.284 ± 0.001 ⁱ	0.96 ± 0.01 ^f	0.782 ± 0.003 ^g	6.2 ± 0.2 ^b	0.29 ± 0.01 ⁱ	0.105 ± 0.008 ^j
C13:0	0.86 ± 0.03 ^f	1.55 ± 0.08 ^c	2.0 ± 0.1 ^b	0.054 ± 0.002 ⁱ	0.041 ± 0.004 ⁱ	0.58 ± 0.01 ^g	2.35 ± 0.04 ^a	1.18 ± 0.04 ^d	0.25 ± 0.01 ^h	1.11 ± 0.06 ^d	0.073 ± 0.001 ⁱ	0.102 ± 0.001 ⁱ	0.047 ± 0.001 ⁱ	0.040 ± 0.003 ⁱ	1.0 ± 0.1 _e	0.25 ± 0.01 ^h
C14:0	1.7 ± 0.2 _c	1.04 ± 0.05 ^{fg}	0.77 ± 0.04 ^h	0.50 ± 0.03 ⁱ	0.9 ± 0.1 ^g	8.2 ± 0.02 ^a	1.11 ± 0.05 ^f	1.59 ± 0.01 ^e	1.84 ± 0.02 ^d	3.86 ± 0.01 ^b	0.98 ± 0.04 ^g	0.765 ± 0.006 ^h	1.68 ± 0.03 ^e	3.50 ± 0.05 ^c	0.96 ± 0.05 ^g	0.9 ± 0.1 _g
C15:0	0.67 ± 0.01 ^{de}	0.80 ± 0.01 ^c	1.23 ± 0.01 ^a	0.59 ± 0.01 ^f	0.88 ± 0.01 ^b	0.16 ± 0.01 ^j	0.79 ± 0.03 ^c	0.54 ± 0.02 ^g	0.49 ± 0.01 ^h	0.60 ± 0.01 ^f	0.68 ± 0.04 ^d	0.160 ± 0.003 ^j	0.59 ± 0.01 ^f	0.640 ± 0.002 ^e	0.51 ± 0.02 ^{gh}	0.317 ± 0.001 ⁱ
C16:0	50 ± 2 ^a	38 ± 1 ^c	43.1 ± 0.1 ^b	22.1 ± 0.2 ^j	27.3 ± 0.9 _h	24.41 ± 0.03 ⁱ	30.5 ± 0.1 ^g	33.9 ± 0.1 ^e	37.05 ± 0.36 ^d	34.3 ± 0.3 ^e	30 ± 1 ^g	12.42 ± 0.04 ^k	32.4 ± 0.4 ^f	34.2 ± 0.1 ^e	27.7 ± 0.1 ^h	27.8 ± 0.3 ^h
C16:1	8.7 ± 0.2 _a	0.627 ± 0.004 ^{fg}	0.94 ± 0.04 ^{de}	0.102 ± 0.005 ^j	0.157 ± 0.001 ^{ij}	0.145 ± 0.001 ^{ij}	0.310 ± 0.001 ^{hi}	3.99 ± 0.08 ^b	3.0 ± 0.2 ^c	2.94 ± 0.03 ^c	0.073 ± 0.001 ^j	0.222 ± 0.005 ^{hij}	0.52 ± 0.01 ^g	0.34 ± 0.01 ^h	0.78 ± 0.01 ^{ef}	1.11 ± 0.03 ^d
C17:0	1.125 ± 0.008 ^b	0.95 ± 0.01 ^c	1.3065 ± 0.0007 ^a	0.47 ± 0.03 ^h	0.58 ± 0.02 ^{fg}	0.15 ± 0.01 ^k	0.426 ± 0.003 ⁱ	0.43 ± 0.01 ⁱ	0.49 ± 0.01 ^h	0.48 ± 0.01 ^h	0.72 ± 0.03 ^e	0.26 ± 0.01 ^j	0.74 ± 0.01 ^{de}	0.60 ± 0.04 ^f	0.764 ± 0.004 ^d	0.55 ± 0.01 ^g
C18:0	11.2 ± 0.5 ^b	6.7 ± 0.4 ^g	5.8 ± 0.2 ^h	3.7 ± 0.2 _{kl}	4.1 ± 0.1 ^{jk}	13.20 ± 0.02 ^a	7.7 ± 0.1 ^f	10.4 ± 0.2 ^c	8.6 ± 0.1 _e	9.29 ± 0.05 ^d	3.41 ± 0.04 ^l	3.51 ± 0.01 ⁱ	4.7 ± 0.1 _i	8.19 ± 0.02 ^e	4.2 ± 0.3 _{jk}	4.44 ± 0.05 ^{ij}
C18:1n9c	3.7 ± 0.2 _m	4.3 ± 0.1 _{lf}	3.54 ± 0.02 ^m	5.2 ± 0.2 ^j	3.75 ± 0.17 ^m	12.86 ± 0.02 ^e	4.7 ± 0.1 _k	5.2 ± 0.1 ^j	6.5 ± 0.1 _i	10.18 ± 0.02 ^f	7.2 ± 0.2 _h	9.6 ± 0.1 _g	20.4 ± 0.2 ^c	14.0 ± 0.3 ^d	28.3 ± 0.2 ^b	48.3 ± 0.2 ^a
C18:2n6c	2.04 ± 0.13 ^j	20 ± 1 ^f	12.9 ± 0.1 _h	46.5 ± 0.8 ^a	39.05 ± 0.42 ^b	8.094 ± 0.005 ⁱ	28.4 ± 0.1 ^c	23 ± 1 ^d	20.1 ± 0.2 ^{ef}	16.6 ± 0.1 ^g	30 ± 1 ^c	8.927 ± 0.001 ⁱ	21.2 ± 0.1 ^e	16.1 ± 0.1 ^g	21 ± 1 ^{ef}	8.87 ± 0.05 ⁱ
C18:3n3	0.22 ± 0.02 ^j	7.4 ± 0.2 _{cd}	2.0 ± 0.2 ^h	10.0 ± 0.3 ^b	11.080 ± 0.004 ^a	1.42 ± 0.02 ⁱ	7.8 ± 0.1 ^c	5.27 ± 0.03 ^e	7.13 ± 0.03 ^d	4.36 ± 0.01 ^f	11.0 ± 0.5 ^a	2.84 ± 0.02 ^g	7.7 ± 0.1 ^c	3.1 ± 0.1 _g	4.129 ± 0.005 ^f	0.27 ± 0.002 ^j
C20:0	4.6 ± 0.2 _a	2.53 ± 0.03 ^c	3.9 ± 0.3 ^b	1.9 ± 0.2 _{def}	2.1 ± 0.1 ^d	0.44 ± 0.01 ^h	1.68 ± 0.03 ^f	1.41 ± 0.01 ^g	2.48 ± 0.05 ^c	1.8 ± 0.1 _{ef}	1.67 ± 0.03 ^f	0.64 ± 0.03 ^h	1.79 ± 0.01 ^{ef}	1.97 ± 0.03 ^{de}	1.70 ± 0.03 ^f	1.33 ± 0.03 ^g
C20:1	2.9 ± 0.3 _a	0.545 ± 0.002 ^c	0.21 ± 0.01 ^{ef}	0.10 ± 0.01 ^{fg}	0.133 ± 0.001 ^{efg}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.12 ± 0.01 ^{fg}	0.388 ± 0.002 ^d	0.26 ± 0.02 ^{de}	0.90 ± 0.02 ^b
C20:2	0.18 ± 0.01 ^e	0.123 ± 0.005 ^{ef}	0.303 ± 0.002 ^d	0.19 ± 0.01 ^e	0.134 ± 0.002 ^{ef}	0.12 ± 0.01 ^{ef}	0.34 ± 0.02 ^d	2.5 ± 0.1 _a	0.46 ± 0.03 ^c	0.6 ± 0.02 ^b	0.131 ± 0.004 ^{ef}	0.17 ± 0.02 ^e	n.d.	0.294 ± 0.004 ^d	0.14 ± 0.01 ^{ef}	0.083 ± 0.003 ^f
C21:0	0.28 ± 0.03 ^d	0.52 ± 0.01 ^b	1.1 ± 0.1 ^a	0.271 ± 0.003 ^{de}	0.250 ± 0.002 ^{def}	n.d.	0.50 ± 0.02 ^b	0.49 ± 0.02 ^b	0.26 ± 0.01 ^{de}	0.29 ± 0.01 ^d	0.37 ± 0.02 ^c	0.12 ± 0.01 ^g	0.21 ± 0.02 ^{ef}	0.28 ± 0.01 ^d	0.413 ± 0.001 ^c	0.195 ± 0.006 ^f
C20:3n6	n.d.	0.15 ± 0.01 ^f	0.0245 ± 0.0007 ^g	0.16 ± 0.01 ^f	0.296 ± 0.003 ^{de}	n.d.	n.d.	n.d.	0.57 ± 0.03 ^b	0.30 ± 0.02 ^d	0.35 ± 0.01 ^c	1.05 ± 0.04 ^a	0.26 ± 0.02 ^c	0.17 ± 0.01 ^f	n.d.	n.d.
C20:3n3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.105 ± 0.005	n.d.	n.d.	n.d.
C22:0	0.99 ± 0.03 ⁱ	2.53 ± 0.04 ^c	4.0 ± 0.3 ^a	2.2 ± 0.1 _d	2.12 ± 0.01 ^{de}	0.38 ± 0.01 ^j	1.9 ± 0.1 _{ef}	1.8 ± 0.1 _{fgh}	2.62 ± 0.01 ^c	1.7 ± 0.1 _{gh}	1.55 ± 0.04 ^h	0.48 ± 0.02 ^j	1.8 ± 0.1 _{fg}	3.00 ± 0.02 ^b	3.2 ± 0.2 ^b	0.075 ± 0.004 ^k
C22:1	0.7 ± 0.1 ^c	0.217 ± 0.003 ^h	0.86 ± 0.02 ^b	0.085 ± 0.004 ^{jk}	0.20 ± 0.01 ^h	0.22 ± 0.01 ^h	0.58 ± 0.02 ^e	0.290 ± 0.005 ^g	0.37 ± 0.02 ^f	n.d.	n.d.	0.63 ± 0.04 ^d	0.137 ± 0.001 ⁱ	0.049 ± 0.005 ^{kl}	0.11 ± 0.01 ^{ij}	1.03 ± 0.03 ^a
C20:5n3	0.384 ± 0.001 ^e	1.37 ± 0.04 ^b	0.822 ± 0.003 ^c	0.23 ± 0.02 ^f	0.238 ± 0.004 ^f	0.25 ± 0.02 ^f	0.46 ± 0.01 ^d	0.14 ± 0.01 ^g	0.38 ± 0.01 ^e	0.412 ± 0.003 ^e	0.059 ± 0.004 ^h	45.5 ± 0.1 ^a	0.20 ± 0.01 ^f	0.021 ± 0.002 ^h	0.41 ± 0.02 ^e	0.062 ± 0.004 ^h
C23:0	0.267 ± 0.004 ^h	1.2 ± 0.1 ^c	2.02 ± 0.04 ^a	0.40 ± 0.02 ^g	0.50 ± 0.01 ^f	0.20 ± 0.01 ^h	0.44 ± 0.02 ^{fg}	0.281 ± 0.003 ^h	0.49 ± 0.02 ^f	1.6 ± 0.1 _b	0.8 ± 0.1 _e	0.52 ± 0.03 ^f	1.2 ± 0.1 _{cd}	0.71 ± 0.02 ^e	1.10 ± 0.02 ^d	0.096 ± 0.004 ⁱ

(continued on next page)

Table 2 (continued)

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	P16
C24:0	1.693 ± 0.002 ^j	3.5 ± 0.1 ^d	2.1 ± 0.1 ^g	2.5 ± 0.1 ^f	2.76 ± 0.02 ^e	0.51 ± 0.01 ⁱ	4.1 ± 0.1 ^c	1.99 ± 0.01 ^{gh}	2.04 ± 0.02 ^{gh}	1.79 ± 0.04 ^{ij}	9.8 ± 0.1 ^a	6.3 ± 0.1 ^b	1.9 ± 0.1 ^m	1.1 ± 0.1 ^k	1.22 ± 0.04 ^k	0.360 ± 0.003 ^l
C24:1	n.d.	2.20 ± 0.05 ^c	7.09 ± 0.02 ^a	0.90 ± 0.04 ^g	1.88 ± 0.04 ^d	0.14 ± 0.01 ^j	1.8 ± 0.1 ^e	0.50 ± 0.03 ⁱ	1.00 ± 0.01 ^f	0.78 ± 0.02 ^h	0.2 ± 0.1 ^j	3.71 ± 0.03 ^b	0.101 ± 0.001 ^j	1.96 ± 0.05 ^d	0.79 ± 0.01 ^h	0.97 ± 0.05 ^{fg}
SFA	81.1 ± 0.5 ^a	63 ± 1 ^d	71.3 ± 0.3 ^c	36.5 ± 0.7 ^m	43 ± 1 ^k	76.76 ± 0.02 ^b	55.6 ± 0.2 ^g	59.2 ± 0.6 ^f	60.6 ± 0.2 ^e	63.82 ± 0.05 ^d	51.5 ± 0.7 ^h	27.33 ± 0.01 ⁿ	49.3 ± 0.2 ⁱ	63.5 ± 0.3 ^d	44 ± 1 ^j	38.4 ± 0.2 ⁱ
MUFA	16.1 ± 0.3 ^e	7.875 ± 0.001 ^k	12.65 ± 0.04 ^h	6.4 ± 0.2 ^m	6.1 ± 0.2 ^m	13.36 ± 0.01 ^g	7.4 ± 0.1 ^l	9.92 ± 0.05 ^j	10.8 ± 0.3 ⁱ	13.91 ± 0.03 ^f	7.5 ± 0.2 ^l	14.2 ± 0.1 ^f	21.3 ± 0.2 ^c	16.7 ± 0.2 ^d	30.2 ± 0.1 ^b	52.3 ± 0.2 ^a
PUFA	2.8 ± 0.2 ^m	29 ± 1 ^g	16.1 ± 0.3 ^k	57.1 ± 0.5 ^b	50.8 ± 0.4 ^c	9.88 ± 0.01 ^l	36.97 ± 0.05 ^e	30.9 ± 0.5 ^f	28.6 ± 0.1 ^g	22.3 ± 0.1 ⁱ	41 ± 1 ^d	58.4 ± 0.1 ^a	29.48 ± 0.02 ^g	19.74 ± 0.03 ^j	25 ± 1 ^h	9.28 ± 0.05 ⁱ

Results are presented as mean ± standard deviation. Different letters correspond to significant differences ($p < 0.05$). Fatty acids are expressed as relative percentage of each fatty acid. dw – dry weight; n.d. – not detected; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids. Identities of fatty acids are indicated in the caption of the Fig. 1.

linolenic acid content in rapeseed oil, which was not confirmed in our study probably because of the different plant tissues studied (seeds vs petioles). On the other hand, Kurasiak-Popowska et al. (2021) reported that the genotypic effect is more profound on fatty acid composition in *Camelina sativa* seed oil than in growing conditions, a finding that also highlights the effect of plant tissue and the genotype.

3.2. Tocopherol, free sugar, and organic acid composition

The qualitative and quantitative composition of the content of tocopherols, organic acids, and free sugars identified in the studied cardoon petioles are presented in Tables 3, 4, and 5, respectively. The α -, β -, and γ -tocopherols were the only isoforms identified, with the α -isoform present in all the harvesting stages analyzed. Samples with low maturation degree (sample P1) or senescent (sample P16) were the ones that showed higher concentrations of this isoform (157 $\mu\text{g}/100\text{ g dw}$ and 32015 $\mu\text{g}/100\text{ g dw}$, respectively). Regarding the β -isoform, it was only identified in samples P11 and P14, while the isoform γ - was identified in five of the sixteen maturation stages studied, especially in the sample P16 where the highest amounts were detected (2760 $\mu\text{g}/100\text{ g dw}$). This study proves the influence of the state of maturation on the composition of the vitamin E isoforms since oscillations were observed in their contents, with statistically significant differences ($p < 0.05$). The highest content of total tocopherols was identified in sample P1 (PGS 1; 32015 $\mu\text{g}/100\text{ g dw}$), and the lowest content was obtained for sample P15 (PGS 8/9; 0.247 $\mu\text{g}/100\text{ g dw}$). Cardoon petioles reveal to be the tissues with the highest variety in tocopherols isoforms compared to the other plant parts studied so far (bracts and seeds) (Maccarone et al., 1999; Mandim, Petropoulos, Giannoulis, Santos-Buelga, et al., 2020; Mandim et al., 2022; Petropoulos, Fernandes, Pereira, et al., 2019), while Chihoub et al. (2019) identified all vitamin E isoforms (α -, β -, γ - and δ -tocopherol) in leaf blades of wild cardoon. Moreover, in contrast to our study Chihoub et al. (2019) suggested δ -tocopherol as the most abundant isoform of vitamin E. As observed in a previous study where tocopherol content in bracts and seeds was reported (Mandim, Petropoulos, Giannoulis, Santos-Buelga, et al., 2020; Mandim et al., 2022), the highest tocopherol content in our study was recorded in samples P16 and P1, which represent the senescence of plants and the formation of new leaves in periods associated with higher temperatures and greater sun exposure (months of August, and September - samples P16 and P1, respectively). According to a study that evaluated the development of grapefruit ripening, genes encoding tocopherol biosynthesis are stimulated with increased fruit ripening, and they are further impaired by the absence of light (Rey, Rodrigo, Diretto, & Zacarias, 2021). These facts agree with the results obtained in the present work. Tocopherols are important biomolecules with antioxidant activity and associated with phyto-remediation processes in plants (Havaux, Eymery, Porfirova, Rey, & Dörmann, 2005), while they are prone to oxidation, which may explain the fluctuation in the observed content throughout the growing season (Mandim, Petropoulos, Giannoulis, Santos-Buelga, et al., 2020). The results obtained suggest that the tocopherol profile varies between parts of the plant and throughout its growth cycle, thus providing a more complete understanding of the species under study and all its bioactive constituents.

Regarding the organic acid's composition, the presence of oxalic, quinic, malic, citric, and fumaric acids was recorded (Table 4). The state of maturation influenced the composition of the organic acids since there was an oscillation with statistically significant differences ($p < 0.05$) in the concentration of organic acids throughout the petiole growth cycle. Petioles harvested at PGS 1 (sample P3) revealed the highest content of organic acids (8 g/100 g dw) compared to the other samples. In contrast, sample P6 (PGS 3/4) has the lowest concentration of organic acids (1.9 g/100 g dw). In general, malic acid was the organic acid with the highest abundance in practically all analyzed samples (1.20–2.53 g/100 g dw), except for samples P2 - P4 and P15, where oxalic acid content stood out (2.1–7.9 g/100 g dw). The profile of

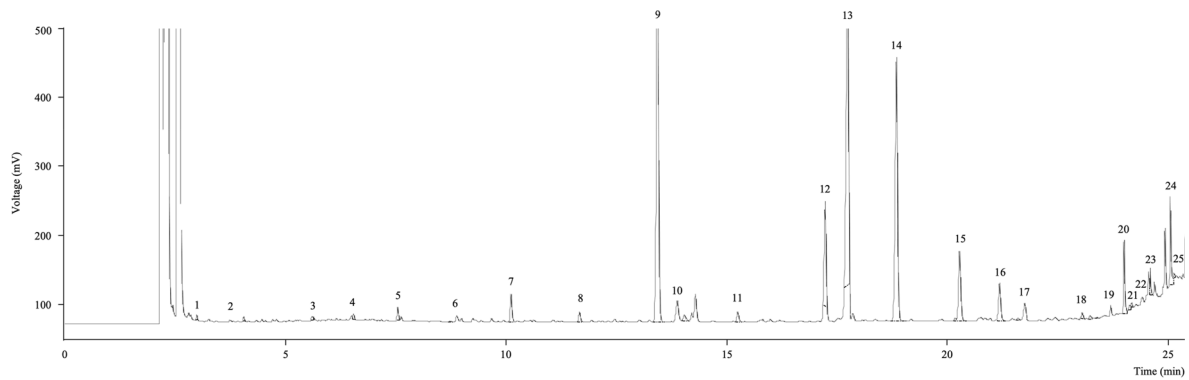


Fig. 1. GC-FID chromatogram of the fatty acids profile of *Cynara cardunculus* petioles (sample P10, PGS 5/6). 1. C6:0 – caproic acid; 2. C8:0 – caprylic acid; 3. C10:0 – capric acid; 4. C11:0 – undecanoic acid; 5. C12:0 – lauric acid; 6. C13:0 – tridecanoic acid; 7. C14:0 – myristic acid; 8. C15:0 – pentadecanoic acid; 9. C16:0 – palmitic acid; 10. C16:1 – palmitoleic acid; 11. C17:0 – heptadecanoic acid; 12. C18:0 – stearic acid; 13. C18:1n9c – oleic acid; 14. C18:2n6c – linoleic acid; 15. C18:3n3 – linolenic acid; 16. C20:0 – arachidic acid; 17. C20:2 – eicosadienoic acid; 18. C21:0 – heneicosanoic acid; 19. C20:3n6 – eicosatrienoic acid; 20. C22:0 – behenic acid; 21. C22:1 – eicosenoic acid; 22. C20:5n3 – eicosapentaenoic acid; 23. C23:0 – tricosanoic acid; 24. C24:0 – lignoceric acid; 25. C24:1 – nervonic acid.

Table 3

Tocopherol's composition of *Cynara cardunculus* L. var. *altilis* petioles in relation to maturity stage.

	Tocopherols (µg per 100 g dw)			
	α-tocopherol	β-tocopherol	γ-tocopherol	Total
P1	32015 ± 22 ^a	n.d.	n.d.	32015 ± 22 ^a
P2	252 ± 8 ^e	n.d.	n.d.	252 ± 8 ^{gh}
P3	374 ± 28 ^c	n.d.	n.d.	375 ± 28 ^e
P4	34 ± 1 ^j	n.d.	n.d.	34 ± 1 ^j
P5	39 ± 1 ^j	n.d.	n.d.	39 ± 1 ^{ij}
P6	41 ± 2 ^j	n.d.	n.d.	41 ± 2 ^{ij}
P7	18.4 ± 0.3 ^j	n.d.	n.d.	18.4 ± 0.3 ^j
P8	33 ± 1 ^j	n.d.	n.d.	33 ± 1 ^j
P9	186 ± 1 ^f	n.d.	360 ± 3 ^b	546 ± 4 ^c
P10	130 ± 1 ^h	n.d.	181 ± 2 ^c	311 ± 3 ^f
P11	31.4 ± 0.3 ^j	120 ± 4 ^a	78 ± 1 ^d	229 ± 4 ^h
P12	67.3 ± 1.5 ⁱ	n.d.	n.d.	67.3 ± 1.5 ⁱ
P13	292 ± 4 ^d	n.d.	178 ± 4 ^c	470 ± 8 ^d
P14	157 ± 3 ^g	118.2 ± 2.5 ^a	n.d.	275 ± 6 ^g
P15	0.247 ± 0.005 ^e	n.d.	n.d.	0.247 ± 0.005 ^{gh}
P16	11193 ± 24 ^b	n.d.	2760 ± 17 ^a	13953 ± 41 ^b

Results are presented as mean ± standard deviation. Different letters correspond to significant differences ($p < 0.05$). dw – dry weight; n.d. – not detected.

organic acids in petioles was similar to that previously described in other parts of plants grown at the same growing conditions, namely seeds (Mandim et al., 2022), heads (Mandim, Petropoulos, Fernandes, et al., 2020), and bracts (Mandim, Petropoulos, Giannoulis, Santos-Buelga, et al., 2020). However, despite the similarities in the detected organic acids, significant differences were observed in the organic acids profile. Therefore, in contrast to what was observed for petioles, where a higher concentration of organic acids was recorded in younger tissues, the remaining plant tissues studied showed a greater abundance at more advanced PGS, e.g., heads at PGS 5/6 (Mandim, Petropoulos, Fernandes, et al., 2020), bracts at PGS 8 (Mandim, Petropoulos, Giannoulis, Santos-Buelga, et al., 2020), and seeds at PGS 6/7 (Mandim et al., 2022). The results of the present study suggest not only the influence that the state of maturation of the petioles has on the composition of organic acids but also the differences in organic acids profile between the various plant tissues of cardoon. Organic acids serve as storage pools of fixed carbon and their biosynthesis is associated with the incomplete oxidation of the products of photosynthesis (Igamberdiev & Eprintsev, 2016). The main organic acids in most plant species are malate and citrate, which participate in the redox balance of cells and can be converted into other organic acids via the tricarboxylic acid cycle (Fridlyand & Scheibe, 1999).

The free sugars identified in cardoon petioles, as well as their

Table 4

Organic acids composition of *Cynara cardunculus* L. var. *altilis* petioles in relation to maturity stage.

	Organic acids (g per 100 g dw)					
	Oxalic	Quinic	Malic	Citric	Fumaric	Total
P1	0.39011 ± 0.00002 ^e	0.21 ± 0.02 ^h	1.587 ± 0.003 ^f	n.d.	n.d.	2.19 ± 0.03 ^{fg}
P2	4.7 ± 0.1 ^b	n.d.	1.90 ± 0.15 ^d	n.d.	n.d.	6.6 ± 0.2 ^b
P3	8 ± 1 ^a	0.139 ± 0.002 ⁱ	n.d.	n.d.	n.d.	8 ± 1 ^a
P4	3.01 ± 0.05 ^c	0.78 ± 0.01 ^c	2.31 ± 0.02 ^b	0.42 ± 0.03 ^a	tr	6.5 ± 0.1 ^b
P5	0.51 ± 0.01 ^e	0.9 ± 0.1 ^b	1.79 ± 0.20 ^e	0.39 ± 0.04 ^a	tr	3.56 ± 0.02 ^c
P6	0.18 ± 0.01 ^e	0.27 ± 0.01 ^h	1.23 ± 0.04 ⁱ	0.24 ± 0.01 ^d	tr	1.9 ± 0.1 ^g
P7	0.19 ± 0.01 ^e	0.38 ± 0.02 ^g	1.65 ± 0.03 ^f	0.08 ± 0.01 ^f	tr	2.30 ± 0.04 ^{fg}
P8	0.19 ± 0.01 ^e	0.61 ± 0.04 ^d	1.30 ± 0.03 ^{hi}	0.0940 ± 0.0004 ^f	tr	2.2 ± 0.1 ^{fg}
P9	0.352 ± 0.001 ^e	0.49 ± 0.02 ^e	1.27 ± 0.03 ^{hi}	0.096 ± 0.001 ^f	tr	2.21 ± 0.04 ^{fg}
P10	0.73 ± 0.01 ^e	0.44 ± 0.02 ^{ef}	1.62 ± 0.05 ^f	0.14 ± 0.01 ^e	tr	2.93 ± 0.03 ^{de}
P11	0.372 ± 0.002 ^e	0.231 ± 0.003 ^h	1.610 ± 0.002 ^f	0.31 ± 0.01 ^c	tr	2.52 ± 0.01 ^{ef}
P12	0.421 ± 0.002 ^e	tr	2.53 ± 0.03 ^a	0.27 ± 0.01 ^d	tr	3.22 ± 0.04 ^{cd}
P13	0.394 ± 0.002 ^e	tr	1.43 ± 0.01 ^g	0.33 ± 0.01 ^{bc}	tr	2.15 ± 0.01 ^{fg}
P14	0.314 ± 0.001 ^e	0.38 ± 0.01 ^{fg}	2.04 ± 0.01 ^c	0.245 ± 0.004 ^d	tr	2.986 ± 0.004 ^{ede}
P15	2.1 ± 0.1 ^d	1.7 ± 0.1	2.0 ± 0.1 ^c	0.35 ± 0.01 ^b	tr	6.15 ± 0.11 ^b
P16	0.51 ± 0.01 ^e	0.14 ± 0.01 ⁱ	1.3589 ± 0.0002 ^{gh}	n.d.	n.d.	2.004 ± 0.004 ^{fg}

Results are presented as mean ± standard deviation. Different letters correspond to significant differences ($p < 0.05$). dw – dry weight; tr – traces; n.d. – not detected.

quantification, are presented in Table 5. As observed for the lipidic content, tocopherols, and organic acids, the free sugar content was also influenced by the maturation state of petioles. Samples P7 (PGS 4) and P5 (PGS 3) had the highest total free sugars content (16.4 g/100 g dw and 16.0 g/100 g dw, respectively), whereas sample P2 (PGS 1) recorded the lowest content (2.51 g/100 g dw). Although fructose, glucose, trehalose, and raffinose were also identified in cardoon petioles, it is the

Table 5Free sugars composition of *Cynara cardunculus* L. var. *altis* petioles in relation to maturity stage.

	Free sugars (g per 100 g dw)					
	Fructose	Glucose	Sucrose	Trehalose	Raffinose	Total
P1	0.145 ± 0.001 ⁱ	0.134 ± 0.005 ^{hi}	2.68 ± 0.01 ⁱ	0.67 ± 0.01 ^e	n.d.	3.63 ± 0.02 ⁱ
P2	0.15 ± 0.01 ⁱ	0.151 ± 0.005 ^{hi}	1.60 ± 0.01 ^k	0.61 ± 0.01	n.d.	2.51 ± 0.03 ^k
P3	0.10 ± 0.01 ^{ij}	0.236 ± 0.005 ^{gh}	1.98 ± 0.02 ^j	0.87 ± 0.02 ^c	n.d.	3.19 ± 0.02 ^j
P4	1.5 ± 0.1 ^e	1.0 ± 0.1 ^d	6.91 ± 0.02 ^d	0.79 ± 0.02 ^d	4.43 ± 0.02 ^a	14.6 ± 0.2 ^b
P5	1.5 ± 0.1 ^e	0.9 ± 0.1 ^d	9.95 ± 0.09 ^a	n.d.	3.6 ± 0.1 ^b	16.0 ± 0.3 ^a
P6	2.68 ± 0.02 ^c	1.9 ± 0.1 ^a	7.9 ± 0.1 ^c	0.42 ± 0.01 ^{fg}	1.7 ± 0.1 ^d	14.60 ± 0.04 ^b
P7	3.0 ± 0.1 ^b	1.7 ± 0.1 ^b	7.0 ± 0.1 ^d	1.0 ± 0.1 ^b	3.7 ± 0.1 ^b	16.4 ± 0.4 ^a
P8	2.10 ± 0.05 ^d	2 ± 1 ^b	4.72 ± 0.01 ^g	1.2 ± 0.1 ^a	1.7 ± 0.1 ^d	11.4 ± 0.4 ^e
P9	3.54 ± 0.15 ^a	1.92 ± 0.03 ^a	5.8 ± 0.1 ^f	n.d.	2.54 ± 0.04 ^c	13.8 ± 0.1 ^c
P10	2.75 ± 0.05 ^c	1.14 ± 0.05 ^c	9.79 ± 0.02 ^{ab}	0.82 ± 0.03 ^{gh}	2.83 ± 0.01 ^e	11.75 ± 0.04 ^b
P11	n.d.	0.169 ± 0.004 ^{hi}	9.8 ± 0.2 ^b	n.d.	0.36 ± 0.01 ^g	10.3 ± 0.2 ^f
P12	0.95 ± 0.01 ^f	0.4 ± 0.1 ^f	9.7 ± 0.1 ^b	0.4 ± 0.1 ^{fg}	0.58 ± 0.05 ^f	12.1 ± 0.1 ^d
P13	0.30 ± 0.05 ^h	0.28 ± 0.03 ^g	3.6 ± 0.01 ^h	0.47 ± 0.04 ^f	n.d.	4.64 ± 0.03 ^h
P14	0.19 ± 0.03 ^{hi}	0.09 ± 0.02 ⁱ	6.3 ± 0.1 ^e	n.d.	n.d.	6.5 ± 0.1 ^g
P15	0.42 ± 0.01 ^g	0.55 ± 0.07 ^{ef}	1.86 ± 0.02 ^j	0.28 ± 0.01 ⁱ	n.d.	3.1 ± 0.1 ^j
P16	0.43 ± 0.01 ^g	0.56 ± 0.01 ^e	2.0 ± 0.1 ^j	0.30 ± 0.02 ^{hi}	n.d.	3.3 ± 0.1 ^{ij}

Results are presented as mean ± standard deviation. Different letters correspond to significant differences ($p < 0.05$). dw – dry weight; n.d. – not detected.

presence of sucrose that stood out, being the major sugar in all analyzed samples (1.60–9.95 g/100 g dw). The highest sucrose content was detected in sample P5 (9.95 g/100 g dw), while fructose and glucose were most abundant in sample P9 (3.54 g/100 g dw and 1.92 g/100 g dw, respectively). Moreover, raffinose and trehalose content was the highest in samples P4 and P8 (4.43 g/100 g dw and 1.2 g/100 g dw, respectively). Previous studies described higher concentrations of free sugars in seeds and bract samples harvested at PGS 6 and 6/7, while they reported sucrose as the main sugar detected (Mandim, Petropoulos, Giannoulis, Santos-Buelga, et al., 2020; Mandim et al., 2022). The fact that in our study the highest contents of free sugars coincide with earlier harvesting stages (PGS 3–4) could be associated with the translocation and accumulation of assimilates (source) in vegetative tissues that could serve as storage pools of carbon (sinks) for the reproductive phase of the plant (Osorio, Ruan, & Fernie, 2014). Moreover, according to Rogers et al. (2005), the lignification process and lignin accumulation are highly associated with sugars availability. Therefore, the increasing trends in sugars content during the reproductive stage followed by a decline at the late growth stages observed in our study could be explained by the lignification of plant tissues when plants enter the maturation and senescence stage which results in sugars consumption.

4. Conclusion

The adequate use of resources is one of the main environmental and economic concerns of our times. In this context, the valorization of crop by-products is necessary to increase the added value of crops while at the same time improving resources use efficiency and reducing the environmental burden. Cardoon petioles are a crop by-product that is usually discarded or used for low added value purposes (e.g., fodder), while they

constitute a large part of the total biomass produced throughout the growth cycle of the species. In this work, the influence that the state of the maturation stage has on the chemical composition of cardoon petioles was proven. Parameters such as the lipidic content and the composition of fatty acids, tocopherols, organic acids, as well as free sugars were influenced by the date of sample collection, thus affecting the potential of alternative uses of petioles in other industrial sectors. In conclusion, our results contribute to a more complete characterization of the species and provide an important source of knowledge, thus allowing the adequate use of resources and the reduction of waste plant material.

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CRediT authorship contribution statement

Filipa Mandim: Methodology, Investigation, Writing – original draft. **Spyridon A. Petropoulos:** Writing – review & editing. **Celestino Santos-Buelga:** Writing – review & editing, Conceptualization, Methodology. **Isabel C.F.R. Ferreira:** Writing – review & editing, Conceptualization, Methodology. **Lillian Barros:** Writing – review & editing, Conceptualization, Methodology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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