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Effect of Harvesting Time on the Chemical Composition of *Cynara cardunculus* L. var. *altilis* Blades

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Abstract: In the present study, the fluctuations in fatty acids, tocopherols, organic acids, and free sugars content of cardoon blades collected at sixteen harvest dates (samples B1–B16, corresponding to principal growth stages (PGS) between 1 and 9) were evaluated. A total of 26 fatty acids were identified, with palmitic (C16:0, 19.9–40.13%), α -linolenic (C18:3n3, 6.39–33.2%), and linoleic (C18:2n6c, 9–34.8%) acids being present in higher relative abundances in most of the samples, while lipid content was the highest in samples of late (B15) and intermediate (B8–B10) stages of maturity. The α - and γ -tocopherols were the only detected vitamin E isoforms, while α -isoform presented the highest concentration (80–8567 $\mu\text{g}/100\text{ g dw}$) in all the studied samples, except for samples B9–B11, for which the γ -tocopherol was detected in higher concentrations. Moreover, samples B1 and B14 showed the highest content of total tocopherols (8352 and 10,197 $\mu\text{g}/100\text{ g dw}$, respectively). The identified organic acids were oxalic, quinic, malic, citric, and fumaric. Malic acid was present in higher concentrations in almost all the samples analyzed, except for samples B3 and B15, in which the presence of oxalic acid stood out. Regarding the free sugar's composition, fructose, glucose, sucrose, trehalose, and raffinose were the only detected compounds, with sucrose being present in higher concentrations in almost all the samples (1.662–10.8 $\text{g}/100\text{ g dw}$), while samples at younger maturation stages, namely samples B4 and B5, presented the highest concentrations of total sugars. In conclusion, the obtained results demonstrate the influence that the growth cycle may have on the chemical composition of this tissue (blades) of the species. Moreover, having a more complete knowledge regarding its composition and identifying the stage of maturation which is most appropriate for obtaining a greater amount of certain bioactive compounds will help to increase the added value of this multi-purpose crop.

Keywords: *Cynara cardunculus* L.; harvest time; growing cycle; principal growth stages; organic acids; fatty acids; free sugars



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1. Introduction

Cardoon, scientifically called *Cynara cardunculus* L. var. *altilis*, is a native plant of the Mediterranean basin, belonging to the *Asteraceae* family and widely consumed due to its medicinal properties and nutritional characteristics. *C. cardunculus* L. also includes two more varieties, namely *Cynara cardunculus* var. *scolymus* (L.) Fiori. (also known as globe or head artichoke) and the wild variable cardoon, also called *Cynara cardunculus* var. *sylvestris* [1,2].

Cardoon, in addition to being used in the making of various dishes, such as soups and salads, is also consumed as a herbal medicine. In traditional medicine, it is used due to its properties as a choleric, anti-hemorrhoid, cardiogenic, antidiabetic agent, and for

the treatment of liver illnesses [3,4]. Several studies have shown the multifaceted bioactive potential of cardoon, and several properties such as antioxidant, anti-inflammatory, cytotoxic, antimicrobial, hypocholesterolemic, and antiviral have been well-established [1,5,6]. Some authors suggest that these properties may be associated with the rich chemical composition of the species, since the various reports highlight the high content of phenolic compounds, namely in phenolic acids derived from caffeoylquinic and dicaffeoylquinic acids, as well as glycosylated flavonoids, namely eriodictyol, luteolin, and apigenin derivatives [4,7,8]. The cardoon is also consumed due to its minerals, carbohydrates, inulin, and high fiber contents [1,9].

In addition to its rich chemical composition and multifaceted medicinal properties, this species shows a great diversity of industrial applications. Its flowers are used as a coagulant agent in the production of Protected Designation of Origin (PDO) cheeses [3], and its stalks are also used in several traditional culinary preparations in some Mediterranean countries. The remaining plant tissues of cardoon, such as bracts, blades, seeds, and roots, are also used for energy production purposes [2]. However, although there are several applications suggested for the different plant tissues of this species, parts such as blades and floral stems continue to be a significant contributor to the huge amounts of by-products generated by the crop [7,10]. Therefore, there is a great potential in the proper characterization of such parts that could allow the exploration of further uses.

Cardoon resistance characteristics to adverse environmental conditions, typical of the climate of Mediterranean basin countries, associated with high performance and low maintenance of the species, are essential factors for stimulating its cultivation and contribute to the increasing interest in cardoon [2,4]. However, factors such as genetic endowment, maturation state, plant tissue, and geographical location, among others, may influence chemical composition and, consequently, the associated potential uses [4,11–13]. The study of the influence that these variables may have on the chemical composition and bioactivities of the species is extremely important and needs further investigation. In addition to contributing to the most complete and deepened knowledge of cardoon, it is essential for the proper use of resources, and simultaneously, for the reduction of the bulk waste material that is generated annually from this crop.

Based on the context of circular economy and sustainability, the present study consisted of the evaluation of the influence of the cardoon blades' growth cycle on their chemical composition. The main aim was to evaluate which stage of the growth cycle is more suitable to collect plant tissues and to identify those applications where they could be used. Thus, more advantageous products and/or applications could be suggested, allowing to reduce the amount of vegetal material wasted and to increase the added value of the crop. Samples collected on sixteen different harvest dates were studied, representing the annual growth cycle of the species. Parameters such as fat content, fatty acids, tocopherols, free sugars, and organic acids were analyzed.

2. Materials and Methods

2.1. Plant Material

The blades of cardoon plants (*Cynara cardunculus* L. var. *altilis* DC cv. *Bianco Avorio*; Fratelli Ignori Spa, Milano, Italy) were collected throughout different phases of a complete growth cycle (regeneration in late September 2017 to senescence in early September of the next year (2018)). Blades were harvested at sixteen harvest dates. According to the Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie (BBCH) scale, the principal growth stages (PGS) of the samples were between 1 and 9. The climatic conditions and sampling were described previously by our team [6,13,14]. Briefly, samples B1, B2, and B3, all at PGS 1, were collected in September, October, and early November, respectively. The sample B4 was collected at the end of November (PGS 2). Samples B5, B6, and B7 were collected in early January (PGS 3), February (PGS 3/4), and March (PGS 4), respectively. Samples B8 (PGS 4/5) and B9 (PGS 5) were collected at the beginning and the end of April, and the samples B10 (PGS 5/6) and B11 (PGS 6) at the beginning and the end of May,

respectively. Sample B12 was collected in June (PGS 6/7). Samples B13 (PGS 7/8) and B14 (PGS 8) were collected at the beginning and the end of July, and finally, the samples B15 (PGS 8/9) and B16 (PGS 9) at the beginning and at the end of August, respectively. The details of harvesting stages and PGS have been previously described in detail by Mandim et al. [15].

After harvest, all samples were properly cleaned with distilled water, dried with soft paper, and stored under freezing conditions. All the samples were lyophilized (Sublimator model EKS, Christian Zirbus Co., Ltd., Harz, Germany), reduced to a fine powder (~20 mesh), and stored in air-sealed bags and under protection from the light (−80 °C) until analysis.

2.2. Analysis of Chemical Composition

2.2.1. Fatty Acids

The lipid fraction of the samples was extracted with petroleum ether with a Soxhlet extraction equipment at 120 °C following the recommended procedure by the Official Methods of Analysis of AOAC [16]. The obtained lipidic fractions of blade samples were subjected to an esterification process and the fatty acid composition was evaluated by gas-liquid chromatography (GC 1000, DANI Instruments, Contone, Gambarogno, Switzerland) coupled to a flame ionization detector (FID) at 260 °C, and with a split/splitless injector. The analytical conditions used were in accordance with the previously described protocol [17]. The identification and quantification were carried out using Clarity DataApex 4.0 software (DataApex, Prague, Czech Republic). The peak retention times of the Fatty Acids Methyl Ester (FAME) of the samples were compared with commercial standards (reference standard mixture 47,885-U; Sigma-Aldrich, St. Louis, MO, USA). The obtained results were presented as relative percentages of each identified fatty acid.

2.2.2. Tocopherols

The tocopherols of cardoon blade samples were extracted according to the procedure described previously [12]. The tocopherols' composition was analyzed by a high-performance liquid chromatography system (HPLC, Knauer, Smartline system 1000, Berlin, Germany) coupled to a fluorescence detector (FP-2020; Jasco, Easton, PA, USA) with an excitation at 290 nm and emission at 330 nm. The analytical conditions used were in accordance with the previously described protocol [18]. Qualitative and quantitative analyses were carried out using the Clarity 2.4 software (DataApex, Podohradská, Czech Republic) and the internal standard (IS, tocol) method, by comparison of the retention times and the obtained spectrum of commercial standards of tocopherols (α -, β -, γ -, and δ -isoforms). The obtained results were expressed in mg per 100 g of dry weight.

2.2.3. Free Sugars

The free sugar content in cardoon blade samples was analyzed by high-performance liquid chromatography (HPLC, Knauer Smartline 2300, Knauer, Berlin, Germany) coupled with a refractive index detector (RI detector; Knauer, Berlin) according to the procedure and analytical conditions previously described by Pires et al. [19]. Qualitative and quantitative analyses were performed with Clarity 2.4 software (DataApex, Prague, Czech Republic), using the internal standard method (IS, melezitose) and by comparison with commercial standards (D-(-)-fructose, D-(+)-sucrose, D-(+)-glucose, D-(+)-trehalose, and D-(+)-raffinose pentahydrate; Sigma-Aldrich, St. Louis, MO, USA). The obtained results were expressed in mg per 100 g of dry weight.

2.2.4. Organic Acids

The organic acids from the blade samples were extracted with a metaphosphoric acid solution (4%) under protection from light, and further analyzed by ultrafast liquid chromatography coupled with a diode array detector (UFLC-PDA, Shimadzu 20A series, Kyoto, Japan), as previously described [18]. The qualitative and quantitative analyses were carried

out using the LabSolutions Multi LC-PDA software (Shimadzu Corporation, Kyoto, Japan) and by comparing the chromatographic information with commercial standards (oxalic, quinic, malic, ascorbic, citric, and fumaric acids, purchased from Sigma- Aldrich, St. Louis, MO, USA) based on the respective calibration curves (oxalic acid— $y = 1 \times 106x + 231,891$, $R^2 = 0.9999$; LOD = 12.55 $\mu\text{g/mL}$, LOQ = 41.82 $\mu\text{g/mL}$; citric acid— $y = 1 \times 105x + 10,277$, $R^2 = 0.9997$; LOD = 10.47 $\mu\text{g/mL}$, LOQ = 31.91 $\mu\text{g/mL}$; quinic acid— $y = 671,557x + 14,583$, $R^2 = 0.9998$; LOD = 24.18 $\mu\text{g/mL}$, LOQ = 80.61 $\mu\text{g/mL}$; malic acid— $y = 950,041x + 6255.6$, $R^2 = 0.9999$, LOD = 35.76 $\mu\text{g/mL}$, LOQ = 119.18 $\mu\text{g/mL}$, and fumaric acid— $y = 1 \times 107 + 614,399$, $R^2 = 0.9986$; LOD = 0.08 $\mu\text{g/mL}$, LOQ = 0.26 $\mu\text{g/mL}$). The obtained results were expressed in mg per 100 g of dry weight.

2.3. Statistical Analysis

All the described assays were performed in triplicate and the obtained results were presented as mean values $\pm$ standard deviation, both calculated using Microsoft Excel. The statistical differences between means were analyzed using an analysis of variance (ANOVA) and the Tukey's honest significant difference test (HSD) ($p < 0.05$) with the SPSS Statistics software (IBM SPSS Statistics for Mac OS, Version 26.0; IBM Corp., Armonk, NY, USA). Before the ANOVA, all samples were tested for normal distribution according to the Shapiro–Wilk's and Levene's tests.

A principal component analysis (PCA) was performed to examine the contribution of each variable to the total diversity and classify the studied maturation stages according to their chemical composition and nutritional value by using the statistical software Statgraphics 5.1.plus (Statpoint Technologies, Inc., Warrenton, VA, USA).

3. Results and Discussion

3.1. Lipidic Fraction and Fatty Acids' Composition

The results regarding the lipidic content of cardoon blades and the fatty acids' composition are presented in Table 1. The allocation of fatty acids in different classes, namely saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA), is also presented in Table 1.

Blade samples in late (B14, PGS 8; 15 mg/100 g dw) and intermediate stages of maturation (samples B8–B10, PGS 4/5, and 5/6; 8.7–10.6 mg/100 g dw, respectively) were those with the highest lipid content. In contrast, samples B3 and B4 of early stages (PGS 1 and 2, respectively) had the lowest lipidic content (2.4 and 1.78 mg/100 g dw, respectively). Moreover, a total of 26 fatty acids were identified in the tested cardoon blades. A representative chromatogram of the fatty acids profile of cardoon blades is shown in Figure 1. Palmitic (C16:0, 19.9–40.13%), α -linolenic (C18:3n3, 6.39–33.2%), and linoleic (C18:2n6c, 9–34.8%) acids showed the highest relative abundance among the identified compounds. Palmitic acid was the one with the highest relative abundance in all samples, except for the samples B2, B9, B11, and B16, in which α -linolenic acid was the most abundant. In contrast, caprylic (C8:0), tridecanoic (C13:0), and tetradecanoic (C14:1) acids were the fatty acids with the lowest relative abundance (0.14–0.799%, 0.088–1.98%, and 0.063–0.15%, respectively). Tetradecanoic acid was identified in only three (samples B4, B11, and B12) of the sixteen samples studied, while for the rest of the fatty acids, a variable content was recorded among the tested samples. Saturated fatty acids (SFA) were the class of fatty acids present in higher abundance in all the samples studied (37.5–69%), except for the samples B9 and B11, in which the presence of polyunsaturated fatty acids (PUFA) stood out (41.9% and 49.46%, respectively).

Table 1. The lipidic fraction content (g/100 g dw) and the fatty acids' composition (%) in cardoon blades collected at different growth stages.

	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15	B16
Lipidic fraction (g/100 g dw)	3.0 ± 0.2 ^g	3.0 ± 0.3 ^g	2.4 ± 0.1 ^h	1.78 ± 0.02 ^h	3.66 ± 0.01 ^g	5.6 ± 0.5 ^e	3.7 ± 0.2 ^g	8.7 ± 0.2 ^c	10.2 ± 0.4 ^b	10.6 ± 0.1 ^b	3.7 ± 0.2 ^g	8.3 ± 0.2 ^c	7.04 ± 0.04 ^d	15 ± 1 ^a	3.2 ± 0.2 ^g	4.7 ± 0.4 ^f
Fatty acids (relative percentage %)																
C6:0	1.151 ± 0.002 ^c	0.46 ± 0.04 ^h	0.48 ± 0.03 ^h	0.30 ± 0.01 ⁱ	0.431 ± 0.001 ^h	0.74 ± 0.01 ^g	1.43 ± 0.01 ^b	1.1 ± 0.1 ^c	0.83 ± 0.03 ^f	0.98 ± 0.03 ^e	0.156 ± 0.002 ^j	0.32 ± 0.03 ⁱ	0.250 ± 0.001 ⁱ	1.7 ± 0.1 ^a	1.1 ± 0.1 ^d	0.83 ± 0.04 ^f
C8:0	0.217 ± 0.005 ⁱ	0.15 ± 0.01 ^j	0.284 ± 0.001 ^{gh}	0.35 ± 0.01 ^{de}	0.15 ± 0.01 ^j	0.325 ± 0.001 ^{ef}	0.44 ± 0.01 ^c	0.366 ± 0.03 ^d	0.31 ± 0.01 ^{fg}	0.26 ± 0.02 ^h	0.144 ± 0.004 ^j	0.621 ± 0.005 ^b	0.3 ± 0.1 ^h	0.799 ± 0.001 ^a	0.20 ± 0.02 ⁱ	0.6 ± 0.1 ^b
C10:0	0.36 ± 0.01 ^e	0.18 ± 0.01 ^j	0.378 ± 0.001 ^e	0.098 ± 0.001 ^k	0.172 ± 0.002 ^j	0.150 ± 0.001 ^j	0.54 ± 0.01 ^c	0.26 ± 0.02 ^{hi}	0.30 ± 0.01 ^g	0.29 ± 0.01 ^{gh}	0.59 ± 0.04 ^b	0.33 ± 0.02 ^f	0.411 ± 0.004 ^d	0.63 ± 0.03 ^a	0.31 ± 0.03 ^{fg}	0.25 ± 0.02 ⁱ
C11:0	0.79 ± 0.02 ^h	0.314 ± 0.004 ^j	0.009 ± 0.001 ^l	0.98 ± 0.05 ^g	1.86 ± 0.02 ^b	1.61 ± 0.05 ^{cd}	1.19 ± 0.02 ^f	1.354 ± 0.02 ^e	1.51 ± 0.02 ^d	1.7 ± 0.1 ^c	0.5 ± 0.2 ⁱ	0.35 ± 0.01 ^j	0.278 ± 0.002 ^j	3.8 ± 0.2 ^a	0.131 ± 0.005 ^k	0.32 ± 0.03 ^j
C12:0	0.025 ± 0.002 ^k	0.19 ± 0.01 ⁱ	0.306 ± 0.003 ^h	0.393 ± 0.001 ^g	0.27 ± 0.02 ^h	1.84 ± 0.01 ^a	1.34 ± 0.01 ^b	1.801 ± 0.1 ^a	0.71 ± 0.04 ^f	0.78 ± 0.03 ^e	0.45 ± 0.02 ^g	0.84 ± 0.01 ^d	0.41 ± 0.03 ^g	1.06 ± 0.03 ^c	0.18 ± 0.01 ^{ij}	0.12 ± 0.01 ^j
C13:0	n.d.	n.d.	n.d.	0.10 ± 0.01 ^e	0.088 ± 0.001 ^e	0.10 ± 0.01 ^e	1.98 ± 0.01 ^a	1.543 ± 0.04 ^c	n.d.	n.d.	1.9 ± 0.1 ^b	n.d.	n.d.	0.61 ± 0.01 ^d	n.d.	n.d.
C14:0	2.1 ± 0.1 ^b	2 ± 1 ^b	0.86 ± 0.04 ^{fg}	1.06 ± 0.01 ^e	1.2 ± 0.1 ^d	1.3 ± 0.1 ^d	2.4 ± 0.2 ^a	1.6 ± 0.1 ^c	1.64 ± 0.05 ^c	0.86 ± 0.02 ^{fg}	0.74 ± 0.03 ^{gh}	1.31 ± 0.01 ^d	0.78 ± 0.03 ^{gh}	1.29 ± 0.05 ^d	0.96 ± 0.01 ^{ef}	0.7 ± 0.1 ^h
C14:1	n.d.	n.d.	n.d.	0.15 ± 0.01 ^a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.063 ± 0.002 ^c	0.13 ± 0.01 ^b	n.d.	n.d.	n.d.	n.d.
C15:0	0.504 ± 0.002 ^d	0.415 ± 0.005 ^f	0.591 ± 0.001 ^c	0.72 ± 0.01 ^a	0.66 ± 0.02 ^b	0.53 ± 0.03 ^d	0.597 ± 0.004 ^c	0.472 ± 0.02 ^e	0.299 ± 0.002 ^g	0.51 ± 0.01 ^d	0.127 ± 0.001 ⁱ	n.d.	0.41 ± 0.02 ^f	0.53 ± 0.01 ^d	0.51 ± 0.05 ^d	0.26 ± 0.03 ^h
C16:0	31.71 ± 0.01 ^{ef}	29 ± 1 ^h	30.76 ± 0.05 ^{efg}	32.97 ± 0.03 ^{cd}	34 ± 2 ^c	30.5 ± 0.2 ^{fg}	31.3 ± 0.3 ^{ef}	25.578 ± 0.1 ⁱ	19.9 ± 0.2 ^j	32.0 ± 0.3 ^{de}	29 ± 1 ^h	40.13 ± 0.05 ^b	24.8 ± 0.2 ⁱ	30.0 ± 0.3 ^{gh}	43 ± 1 ^a	25.2 ± 0.4 ⁱ
C16:1	n.d.	2.2 ± 0.1 ^a	0.147 ± 0.004 ^h	0.860 ± 0.003 ^e	0.97 ± 0.04 ^{cd}	2.12 ± 0.05 ^a	0.5 ± 0.05 ^f	0.612 ± 0.02 ^f	0.6 ± 0.1 ^f	1.0 ± 0.1 ^c	1.4 ± 0.1 ^b	1.05 ± 0.01 ^c	0.90 ± 0.02 ^{de}	0.835 ± 0.001 ^e	0.39 ± 0.03 ^g	n.d.
C17:0	0.78 ± 0.01 ^d	0.60 ± 0.03 ^f	0.828 ± 0.004 ^c	0.77 ± 0.03 ^d	0.59 ± 0.03 ^f	0.61 ± 0.03 ^f	0.62 ± 0.01 ^f	0.601 ± 0.02 ^f	0.30 ± 0.01 ⁱ	0.45 ± 0.01 ^h	0.52 ± 0.02 ^g	1.099 ± 0.002 ^a	0.77 ± 0.03 ^d	1.005 ± 0.004 ^b	0.7 ± 0.1 ^e	0.54 ± 0.01 ^g
C18:0	6.825 ± 0.001 ^e	6.2 ± 0.5 ^f	7.69 ± 0.04 ^{cd}	6.56 ± 0.05 ^{ef}	9 ± 1 ^b	8.84 ± 0.02 ^b	9.01 ± 0.01 ^b	11.1 ± 0.1 ^a	6.89 ± 0.05 ^e	8.0 ± 0.1 ^c	3.9 ± 0.4 ^h	7.392 ± 0.005 ^d	5.3 ± 0.3 ^g	7.7 ± 0.1 ^{cd}	8.1 ± 0.3 ^c	6.4 ± 0.1 ^f
C18:1n9c	3.951 ± 0.003 ^{jk}	2.71 ± 0.04 ^m	3.13 ± 0.01 ^l	4.5 ± 0.5 ^{hi}	2.2 ± 0.1 ⁿ	6.9 ± 0.1 ^e	13.28 ± 0.02 ^c	5.3 ± 0.1 ^f	19.1 ± 0.2 ^b	2.3 ± 0.2 ⁿ	3.8 ± 0.4 ^k	7.30 ± 0.01 ^d	4.3 ± 0.4 ^{ij}	4.8 ± 0.1 ^{gh}	4.99 ± 0.02 ^{fg}	23.0 ± 0.3 ^a
C18:2n6c	15.60 ± 0.05 ^f	14 ± 1 ^h	17.56 ± 0.02 ^e	19.8 ± 0.5 ^d	19.9 ± 0.4 ^d	17.4 ± 0.1 ^e	17.2 ± 0.1 ^e	10.8 ± 0.1 ^j	34.8 ± 0.2 ^a	13.3 ± 0.1 ⁱ	14.7 ± 0.1 ^g	21.75 ± 0.03 ^c	14.0 ± 0.1 ^h	17.15 ± 0.04 ^e	9 ± 1 ^k	27.5 ± 0.4 ^b
C18:3n3c	22.89 ± 0.03 ^c	33 ± 1 ^a	23.68 ± 0.03 ^c	17.4 ± 0.5 ^f	22 ± 1 ^d	21.2 ± 0.3 ^d	7.29 ± 0.04 ⁱ	15.5 ± 0.1 ^g	6.39 ± 0.05 ^j	25.17 ± 0.02 ^c	33.2 ± 0.2 ^a	n.d.	20.3 ± 0.3 ^e	13.4 ± 0.2 ^h	16 ± 1 ^g	6.8 ± 0.1 ^{ij}
C20:0	4.17 ± 0.04 ^d	3.6 ± 0.2 ^e	5.90 ± 0.01 ^a	5.0 ± 0.1 ^c	2.52 ± 0.02 ^g	1.96 ± 0.04 ^h	1.9 ± 0.1 ^h	5.821 ± 0.3 ^a	1.23 ± 0.01 ⁱ	2.52 ± 0.02 ^g	2.8 ± 0.1 ^f	5.488 ± 0.004 ^b	5.9 ± 0.2 ^a	2.4 ± 0.1 ^g	5.3 ± 0.2 ^b	1.83 ± 0.03 ^h
C20:1	n.d.	0.08 ± 0.01 ^e	0.115 ± 0.001 ^d	0.128 ± 0.004 ^c	0.088 ± 0.004 ^e	n.d.	n.d.	n.d.	0.16 ± 0.02 ^b	n.d.	n.d.	n.d.	0.11 ± 0.01 ^d	n.d.	n.d.	0.70 ± 0.02 ^a
C20:2	n.d.	0.162 ± 0.001 ^b	0.264 ± 0.002 ^a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
C21:0	0.54 ± 0.02 ^f	0.56 ± 0.02 ^f	1.05 ± 0.04 ^a	0.8 ± 0.1 ^d	0.34 ± 0.03 ^h	0.345 ± 0.004 ^h	0.253 ± 0.004 ⁱ	0.688 ± 0.02 ^e	0.09 ± 0.01 ^j	0.43 ± 0.04 ^g	0.323 ± 0.003 ^h	0.81 ± 0.01 ^c	0.6 ± 0.1 ^f	n.d.	0.862 ± 0.004 ^b	0.26 ± 0.02 ⁱ
C20:3n3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.3 ± 0.1 ^c	1.389 ± 0.05 ^c	0.34 ± 0.01 ^d	n.d.	1.4 ± 0.1 ^c	3.13 ± 0.04 ^b	3.8 ± 0.2 ^a	n.d.	n.d.	n.d.
C22:0	4.9 ± 0.1 ^c	3.4 ± 0.3 ^g	4.53 ± 0.05 ^d	3.8 ± 0.1 ^f	2.51 ± 0.04 ⁱ	1.19 ± 0.03 ^l	3.2 ± 0.1 ^g	4.199 ± 0.03 ^e	1.74 ± 0.01 ^k	2.20 ± 0.01 ^j	2.8 ± 0.1 ^h	5.27 ± 0.01 ^b	6.8 ± 0.1 ^a	3.4 ± 0.1 ^g	4.9 ± 0.4 ^c	2.1 ± 0.1 ^j
C22:1	0.52 ± 0.02 ^f	0.28 ± 0.03 ^g	0.105 ± 0.003 ⁱ	0.096 ± 0.001 ⁱ	0.22 ± 0.01 ^{gh}	0.18 ± 0.02 ^{hi}	0.26 ± 0.02 ^{gh}	0.221 ± 0.01 ^{gh}	0.9 ± 0.1 ^e	1.53 ± 0.02 ^c	0.13 ± 0.01 ⁱ	0.53 ± 0.02 ^f	6.9 ± 0.1 ^a	5.5 ± 0.1 ^b	n.d.	1.2 ± 0.1 ^d
C20:5n3	0.604 ± 0.001 ^c	0.16 ± 0.01 ^{fg}	0.29 ± 0.02 ^{de}	0.051 ± 0.003 ^{gh}	0.21 ± 0.01 ^{ef}	0.27 ± 0.03 ^{def}	0.791 ± 0.005 ^b	0.34 ± 0.02 ^d	0.375 ± 0.02 ^d	0.63 ± 0.04 ^c	0.15 ± 0.01 ^{fg}	0.30 ± 0.03 ^{de}	2.6 ± 0.3 ^a	0.77 ± 0.05 ^b	n.d.	0.060 ± 0.001 ^{gh}
C23:0	0.62 ± 0.01 ^j	0.182 ± 0.001 ^l	0.929 ± 0.003 ^{gh}	0.9 ± 0.1 ^{hi}	0.273 ± 0.002 ^k	0.84 ± 0.03 ⁱ	1.8 ± 0.1 ^c	7.0 ± 0.1 ^a	1.06 ± 0.04 ^{ef}	2.8 ± 0.1 ^b	0.63 ± 0.01 ^j	1.37 ± 0.05 ^d	0.260 ± 0.001 ^{kl}	1.10 ± 0.01 ^e	0.99 ± 0.04 ^{fg}	0.245 ± 0.003 ^{kl}
C24:0	1.76 ± 0.01 ^d	0.09 ± 0.01 ^m	0.11 ± 0.01 ^{lm}	2.221 ± 0.004 ^c	1.04 ± 0.03 ^h	0.94 ± 0.02 ⁱ	1.3 ± 0.1 ^f	2.454 ± 0.005 ^a	0.59 ± 0.04 ^j	2.31 ± 0.01 ^b	0.166 ± 0.001 ^l	0.52 ± 0.02 ^k	n.d.	1.5 ± 0.1 ^e	1.48 ± 0.02 ^e	1.19 ± 0.03 ^g
SFA	56.44 ± 0.04 ^{de}	47.6 ± 2 ^h	54.70 ± 0.05 ^f	56.92 ± 0.05 ^{de}	55 ± 1 ^f	51.8 ± 0.1 ^g	59.35 ± 0.2 ^c	65.9 ± 0.1 ^b	37.5 ± 0.1 ^k	56.0 ± 0.1 ^e	45.2 ± 0.3 ⁱ	65.84 ± 0.03 ^b	47 ± 1 ^h	57.5 ± 0.3 ^d	69 ± 1 ^a	41 ± 1 ^j
MUFA	4.47 ± 0.02 ^k	5.2 ± 0.1 ⁱ	3.50 ± 0.01 ^l	5.76 ± 0.05 ^h	3.5 ± 0.1 ^l	9.2 ± 0.1 ^f	14.1 ± 0.1 ^c	6.1 ± 0.1 ^g	20.7 ± 0.2 ^b	4.9 ± 0.2 ^j	5.4 ± 0.2 ⁱ	8.997 ± 0.004 ^f	12.2 ± 0.3 ^d	11.16 ± 0.05 ^e	5.4 ± 0.1 ⁱ	24.9 ± 0.2 ^a
PUFA	39.09 ± 0.02 ^d	47 ± 2 ^b	41.8 ± 0.1 ^c	37.32 ± 0.01 ^e	41.9 ± 0.6 ^c	38.9 ± 0.2 ^d	26.6 ± 0.1 ⁱ	27.9 ± 0.1 ^h	41.9 ± 0.2 ^c	39.1 ± 0.1 ^d	49.46 ± 0.01 ^a	25.17 ± 0.03 ^j	41 ± 1 ^c	31.3 ± 0.2 ^g	25 ± 1 ^j	34.3 ± 0.4 ^f

C6:0—caproic acid; C8:0—caprylic acid; C10:0—capric acid; C11:0—undecanoic acid; C12:0—lauric acid; C13:0—tridecanoic acid; C14:0—myristic acid; C14:1—tetradecanoic acid; C15:0—pentadecanoic acid; C16:0—palmitic acid; C16:1—palmitoleic acid; C17:0—heptadecanoic acid; C18:0—stearic acid; C18:1n9—oleic acid; C18:2n6—linoleic acid; C18:3n3—alpha-linolenic acid; C20:0—arachidic acid; C20:1—gondoic acid; C20:2—eicosadienoic acid; C21:0—heneicosanoic acid; C20:3n3—11,14,17-eicosatrienoic acid; C22:0—behenic acid; C22:1—erucic acid; C20:5n3—eicosapentaenoic acid; C23:0—tricosanoic acid; C24:0—lignoceric acid; SFA—saturated fatty acids; MUFA—monounsaturated fatty acids; PUFA—polyunsaturated fatty acids. Means followed by different Latin letters in the same row are significantly different according to Tukey's HSD test ($p = 0.05$).

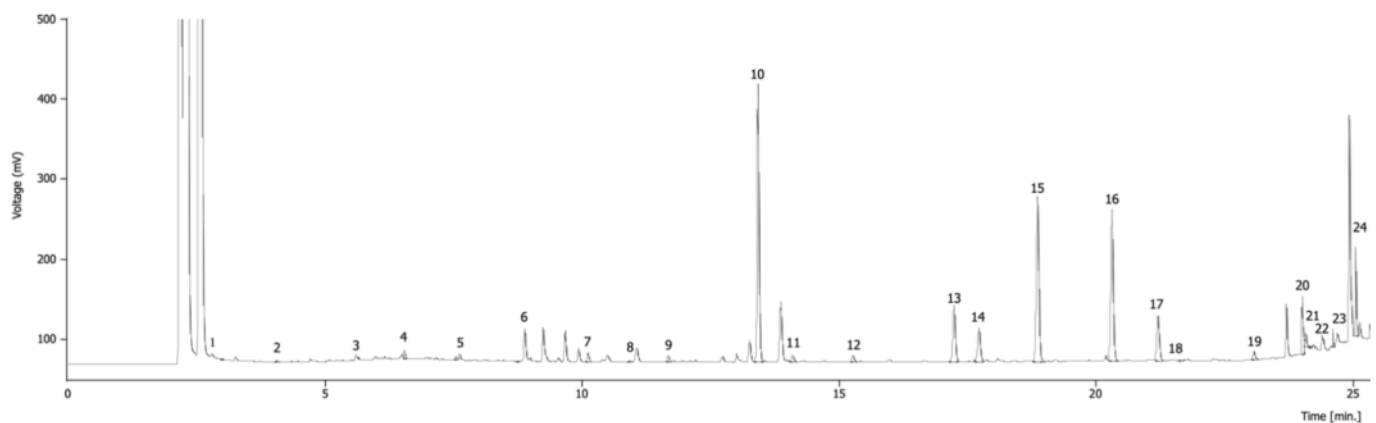


Figure 1. Fatty acids chromatogram of *Cynara cardunculus* L. var. *altilis* blades (sample B4, PGS 2). 1. C6:0—caproic acid; 2. C8:0—caprylic acid; 3. C10:0—capric acid; 4. C11:0—undecanoic acid; 5. C12:0—lauric acid; 6. C13:0—tridecanoic acid; 7. C14:0—myristic acid; 8. C14:1—tetradecanoic acid; 9. C15:0—pentadecanoic acid; 10. C16:0—palmitic acid; 11. C16:1—palmitoleic acid; 12. C17:0—heptadecanoic acid; 13. C18:0—stearic acid; 14. C18:1n9c—oleic acid; 15. C18:2n6c—linoleic acid; 16. C18:3n3—linolenic acid; 17. C20:0—arachidic acid; 18. C20:1—gadoleic acid; 19. C21:0—heneicosanoic 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.

The obtained results demonstrated that the maturation stage of cardoon blades influenced the lipid composition. Previous studies of our team have evaluated the influence of maturity on different plant tissues of cardoon (e.g., seeds, bracts, floral capitula, and petioles) and suggested a variable effect depending on the plant part and the growth stage [12,13,15,20]. The analyzed samples in those reports were all collected from the same experimental plants grown under the same edaphoclimatic conditions, which allows the direct comparison with the results presented herein. Statistically significant differences were also observed in the lipid content of other cardoon tissues. In particular, the analysis of the lipid composition of different cardoon plant tissues verified the higher lipid content in samples collected at intermediate stages of maturation, e.g., samples of bracts at PGS 5/6 [20], floral capitula at PGS 5 [13], and petioles at PGS 6/7 [14]. Similar results were reported by Curt et al. [21], who also noted the significant effect of the growing condition on the fatty acid composition of cardoon seed oil, as well by Ashrafi and Razmjoo [22] and Zemour et al. [23], who studied the fatty acid composition of safflower seeds. According to Sanyal and Linder [24], the proportion of saturated and unsaturated fatty acids is adapted according to the ambient temperatures to increase fitness as part of plants' adaptive evolution.

Regarding the individual fatty acids' content, palmitic, linoleic, and linolenic acids were also the fatty acids with the highest relative abundance in other cardoon tissues studied (e.g., bracts, heads, and petioles), although a variable fatty acids profile was recorded [13,14,20]. For example, the content of palmitic acid (C16:0) in bracts ranged between 0.95% and 44%, stearic acid (C18:0) between 6.64% and 44.37%, oleic acid (C18:1n9c) between 4.16% and 29.0%, and eicosatrienoic acid (C18:2n6c) between 2.27% and 16.852% [13], whereas in petioles, palmitic acid (C16:0) had the highest relative abundance, 12.42–50% (in all sampling dates), as well as linoleic acid (C18:2n6c), the content of which was 46.5% and 39.05% in early maturity stages [14]. Finally, palmitic (C16:0; 14.62–43.8%), oleic (C18:1n9c; 4.48–46.6%), and linoleic (C18:2n6c; 0.748–30.6%) acids were detected in higher abundance in cardoon heads harvested at different maturity stages [20]. Although the same fatty acids are very common in other plant species [25], there are the proportions of PUFA/SFA and n6/n3 (values higher than 0.45 and lower than 4.0, respectively, are associated with good nutritional quality) that are important from a nutritional point of view; in this respect, favorable ratios are found in several species that are part of the Mediterranean diet, such

as cardoon [6,26]. Indeed, the results of this study indicate that cardoon blades have a satisfactory nutritious lipidic profile at most maturation stages, except in samples B7, B8, B12, and B15, where the PUFA/SFA ratio was lower than 0.45, and samples B9, B12, and B16, where the n6/n3 ratio was higher than 5. Considering that fatty acids serve as energy stores, the fluctuations in lipidic fraction, as well as the variation in fatty acids' composition throughout the growth cycle, could be associated with plant energy needs at specific harvesting stages and growing conditions [27]. Another aspect to be considered is that fatty acids are integral parts of cell membranes, and therefore any changes in their composition could be related with the signaling of plant defense mechanisms against environmental stressors [28].

3.2. Tocopherols, Free Sugars, and Organic Acid Content

The obtained results regarding the composition of tocopherols are presented in Table 2. The α - and γ -tocopherols were the only isoforms identified. The α -isoform was present in the highest concentration in all samples studied (80–6637 $\mu\text{g}/100\text{ g dw}$), except for samples B9–B11, for which the γ -tocopherol was detected in higher concentrations (399–1478 $\mu\text{g}/100\text{ g dw}$). The obtained results reveal that the content of the different tocopherol isoforms varies significantly ($p < 0.05$) throughout the development of the species. Samples B1 and B14 (PGS 1 and 8, respectively) showed the highest content of total tocopherols (8352 and 10,197 $\mu\text{g}/100\text{ g dw}$, respectively), whereas samples B7 and B8 were those with the lowest concentration (115.7 and 99 $\mu\text{g}/100\text{ g dw}$, respectively). Moreover, a fluctuating pattern in total tocopherols content was observed, with high contents recorded at the regeneration stage (B1 sample) followed by a gradual decrease up to sample B8 (PGS 4/5); then, the content increased up to the senescence stage (B14; PGS 8), followed by a decrease in the samples B15 and B16 (PGS 9). This pattern was also detected in petioles by Mandim et al. [14], attributing the fluctuations to physiological processes at the corresponding growth stages, as well as to environmental conditions (air temperature and sunlight exposure), which may cause stressful conditions and result in the oxidation of protective molecules such as tocopherols [13,29,30]. The increased content of tocopherols in senescing leaves can be explained by the upregulation of specific genes which lead to its accumulation [31], while the increased content at regeneration could be assigned to the antioxidant activities of tocopherols and in plant adaptation mechanisms to environmental conditions [31,32].

Table 2. Tocopherols of *Cynara cardunculus* L. blades collected at different growth stages.

	Tocopherols ($\mu\text{g per } 100\text{ g dw}$)		
	α -Tocopherol	γ -Tocopherol	Total
B1	6637 $\pm$ 45 ^b	1715 $\pm$ 96 ^c	8352 $\pm$ 142 ^b
B2	5145 $\pm$ 38 ^c	n.d.	5145 $\pm$ 38 ^d
B3	2254 $\pm$ 24 ^e	1174 $\pm$ 47 ^f	3428 $\pm$ 72 ^g
B4	146 $\pm$ 4 ^h	n.d.	146 $\pm$ 1 ^l
B5	129 $\pm$ 2 ^h	n.d.	129 $\pm$ 2 ^l
B6	145.0 $\pm$ 0.5 ^h	n.d.	145.0 $\pm$ 0.5 ^l
B7	115.7 $\pm$ 0.4 ^{hi}	n.d.	115.7 $\pm$ 0.4 ^l
B8	99 $\pm$ 1 ^{ij}	n.d.	99 $\pm$ 1 ^l
B9	80.0 $\pm$ 2 ^j	399 $\pm$ 6 ^h	478.8 $\pm$ 4.5 ^k
B10	135.5 $\pm$ 1.5 ^h	554 $\pm$ 3 ^g	689.5 $\pm$ 4.8 ^j
B11	420 $\pm$ 3 ^g	1478 $\pm$ 2 ^e	1897.5 $\pm$ 0.9 ^h
B12	1834 $\pm$ 2 ^f	1675.5 $\pm$ 3.0 ^c	3509 $\pm$ 5 ^f
B13	3912 $\pm$ 4 ^d	2181.3 $\pm$ 0.4 ^b	6093.7 $\pm$ 3.5 ^c
B14	8567 $\pm$ 4 ^a	1630.5 $\pm$ 4.5 ^d	10,197 $\pm$ 1 ^a
B15	2256.8 $\pm$ 6.5 ^k	2002 $\pm$ 4 ^a	4263 $\pm$ 10 ^e
B16	966 $\pm$ 3 ^k	n.d.	966 $\pm$ 3 ⁱ

Results are presented as mean $\pm$ standard deviation. Different letters in the same column correspond to significant differences ($p < 0.05$). dw—dry weight; n.d.—not detected.

The results regarding the free sugars identified in cardoon blades are presented in Table 3. The sugars identified were fructose, glucose, sucrose, trehalose, and raffinose. Sucrose was the most abundant sugar detected in practically all samples studied (1.662–10.8 g/100 g dw). Although, the sugar content varied throughout the development of the species, with samples in more advanced stages of development (particularly sample B15) showing the lowest sugar content (2.9 g/100 g dw). In turn, younger maturation stages, namely samples B4 and B5 (PGS 2 and PGS 3, respectively), presented the highest concentrations of sugars (15.5 and 16.87 g/100 g dw). This fact agrees with the results observed for other plant tissues of cardoon, namely in the floral capitula, bracts, and petioles [13,14,20]. The transition through the different stages of the species development requires large amounts of energy, which justifies the low sugar content recorded at late growth stages. Moreover, the low sugar content detected at senescence and at the regeneration stage (B15, B16, and B1, respectively) preceded by high contents (e.g., B13) could be partly associated with the decreasing area of photosynthetic tissues which are responsible for the biosynthetic processes. Besides, according to Izumi and Ishida [33], sugar accumulation serves as signaling for leaves' senescence through the production of Rubisco-containing spherical bodies, which mobilize chloroplasts in the vacuole. Several studies report that species store different classes of carbon compounds, namely sugars and organic acids, used as metabolic reserves to guarantee the energy needs associated with these stages of development [34]. The lignification process associated with the late stages of the species growth cycle, which involves the formation of lignin carbohydrate complexes, could also justify the lower sugar content observed in cardoon blades [35]. In addition to the maturation cycle, parameters such as the geographic location and the genetic information of the species may also have an influence on the composition of free sugars. A study carried out by Petropoulos et al. [36] found differences between different artichoke and cardoon genotypes, reporting the effect that the genotype and growing conditions may have on the chemical composition of heads.

Table 3. Free sugars identified on *Cynara cardunculus* L. blades collected at different growth stages.

Free Sugars (g per 100 g dw)						
	Fructose	Glucose	Sucrose	Trehalose	Raffinose	Total
B1	0.79 ± 0.02 ^b	0.61 ± 0.03 ^a	2.18 ± 0.02 ^m	0.33 ± 0.01 ^h	n.d.	3.91 ± 0.02 ^m
B2	0.61 ± 0.02 ^d	0.38 ± 0.01 ^b	3.97 ± 0.01 ^j	0.26 ± 0.01 ⁱ	n.d.	5.22 ± 0.03 ^k
B3	n.d.	0.25 ± 0.02 ^{de}	1.662 ± 0.004 ^o	1.68 ± 0.02 ^b	n.d.	3.590 ± 0.001 ⁿ
B4	0.53 ± 0.05 ^e	0.06 ± 0.03 ^j	6.1 ± 0.1 ^f	2.0 ± 0.1 ^a	6.799 ± 0.002 ^b	15.5 ± 0.1 ^b
B5	0.68 ± 0.03 ^c	0.18 ± 0.04 ^{fg}	6.45 ± 0.05 ^e	1.64 ± 0.03 ^b	7.9 ± 0.1 ^a	16.87 ± 0.03 ^a
B6	0.85 ± 0.02 ^a	0.30 ± 0.01 ^{cd}	5.9 ± 0.1 ^h	2.0 ± 0.1 ^a	4.3 ± 0.1 ^c	13.4 ± 0.1 ^c
B7	0.9 ± 0.1 ^a	0.23 ± 0.05 ^{ef}	4.1 ± 0.1 ⁱ	1.3 ± 0.1 ^c	3.17 ± 0.01 ^d	9.80 ± 0.01 ^h
B8	0.58 ± 0.02 ^d	0.1 ± 0.1 ⁱ	3.7 ± 0.1 ^k	0.8 ± 0.1 ^d	2.7 ± 0.1 ^f	7.9 ± 0.2 ⁱ
B9	0.76 ± 0.05 ^b	0.34 ± 0.03 ^{bc}	6.1 ± 0.2 ^{fg}	0.67 ± 0.02 ^e	2.67 ± 0.03 ^f	10.5 ± 0.2 ^g
B10	0.47 ± 0.01 ^f	0.29 ± 0.05 ^{cd}	7.34 ± 0.02 ^d	0.82 ± 0.03 ^d	2.83 ± 0.01 ^e	11.75 ± 0.04 ^e
B11	0.51 ± 0.01 ^{ef}	0.19 ± 0.05 ^{efgh}	8.72 ± 0.04 ^c	0.42 ± 0.05 ^g	1.3 ± 0.1 ^g	11.12 ± 0.02 ^f
B12	0.25 ± 0.05 ^g	0.59 ± 0.01 ^a	9.4 ± 0.1 ^b	0.57 ± 0.05 ^f	1.5 ± 0.1 ^g	12.0 ± 0.2 ^d
B13	0.17 ± 0.01 ^h	0.14 ± 0.03 ^{hi}	6.0 ± 0.1 ^{gh}	0.185 ± 0.003 ^j	n.d.	6.45 ± 0.12 ^j
B14	0.16 ± 0.01 ^h	0.16 ± 0.02 ^{ghi}	10.8 ± 0.1 ^a	n.d.	n.d.	11.08 ± 0.05 ^f
B15	0.17 ± 0.01 ^h	0.168 ± 0.002 ^{fg}	2.01 ± 0.04 ⁿ	0.585 ± 0.003 ^f	n.d.	2.9 ± 0.1 ^o
B16	0.27 ± 0.01 ^g	0.21 ± 0.01 ^{efg}	2.93 ± 0.01 ^l	0.70 ± 0.01 ^e	n.d.	4.12 ± 0.03 ^l

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

Regarding the content of organic acids, the results are presented in Table 4. The presence of oxalic, quinic, malic, citric, and fumaric acids was detected. Malic acid was present in greater amounts in all stages of maturation studied (0.631–6.16 g/100 g dw), except for samples B3 (PGS 1) and B15 (PGS 8/9), in which the presence of oxalic acid stood

out. The sample with the highest concentration of oxalic and total organic acids was B15 (PGS 8/9), with a content of 40.8 and 41.8 g/100 g dw, respectively, while the sample with the lowest content of total organic acids was B16 (PGS 9) with 1.15 g/100 g dw. Previous studies carried out with different cardoon plant tissues described a composition in organic acids similar to that observed for blades. In particular, Mandim et al. [14] found a similar pattern in total organic acid content where the highest amounts were recorded at similar growth stages (PGS 1 and PGS 8/9), mostly due to the high oxalic acid content. These findings could be associated with growing conditions, which were similar in both studies. Additionally, in most of the cardoon tissues studied, samples in the intermediate stages of maturation had higher levels of organic acids [13,20], contrary to what was observed in petioles, which presented higher concentrations in younger tissues [14].

Table 4. Organic acids identified on *Cynara cardunculus* L. blades collected at different growth stages.

	Organic Acids (g per 100 g dw)					Total
	Oxalic	Quinic	Malic	Citric	Fumaric	
B1	0.4 ± 0.1 ^{cd}	0.391 ± 0.003 ^j	0.81 ± 0.05 ^l	n.d.	n.d.	1.62 ± 0.02 ^j
B2	0.86 ± 0.03 ^c	0.355 ± 0.004 ^k	1.06 ± 0.02 ^k	n.d.	n.d.	2.28 ± 0.01 ⁱ
B3	8 ± 1 ^b	0.139 ± 0.002 ⁿ	tr	n.d.	n.d.	8 ± 1 ^c
B4	0.79 ± 0.02 ^{cd}	1.17 ± 0.01 ^f	2.22 ± 0.02 ^j	1.1 ± 0.1 ^e	tr	5.3 ± 0.1 ^f
B5	0.250 ± 0.003 ^d	1.29 ± 0.01 ^d	2.39 ± 0.04 ⁱ	0.86 ± 0.02 ^h	tr	4.8 ± 0.1 ^g
B6	0.275 ± 0.003 ^d	1.23 ± 0.02 ^e	3.03 ± 0.02 ^g	0.89 ± 0.04 ^{gh}	tr	5.4 ± 0.1 ^f
B7	0.3441 ± 0.0001 ^{cd}	1.73 ± 0.04 ^b	2.80 ± 0.01 ^h	0.75 ± 0.02 ⁱ	0.011 ± 0.001 ^b	5.63 ± 0.05 ^f
B8	0.76 ± 0.02 ^{cd}	2.54 ± 0.03 ^a	3.5 ± 0.1 ^d	1.09 ± 0.03 ^e	0.014 ± 0.001 ^a	7.9 ± 0.2 ^{cd}
B9	0.53 ± 0.01 ^{cd}	1.4 ± 0.1 ^c	3.3 ± 0.2 ^e	1.00 ± 0.04 ^f	0.0100 ± 0.0004 ^c	6.3 ± 0.3 ^e
B10	0.55 ± 0.01 ^{cd}	0.95 ± 0.01 ^g	4.18 ± 0.02 ^b	1.8 ± 0.1 ^b	0.0041 ± 0.0002 ^d	7.5 ± 0.2 ^d
B11	0.490 ± 0.003 ^{cd}	0.64 ± 0.02 ^h	3.21 ± 0.03 ^f	2.4 ± 0.1 ^a	tr	6.8 ± 0.1 ^e
B12	0.75 ± 0.01 ^{cd}	0.51 ± 0.01 ⁱ	6.16 ± 0.02 ^a	1.88 ± 0.03 ^b	0.0016 ± 0.0001 ^e	9.31 ± 0.02 ^b
B13	0.392 ± 0.001 ^{cd}	0.30 ± 0.01 ^l	3.79 ± 0.01 ^c	1.25 ± 0.05 ^d	tr	5.73 ± 0.03 ^f
B14	0.549 ± 0.002 ^{cd}	0.185 ± 0.001 ^m	2.231 ± 0.001 ^j	1.34 ± 0.04 ^c	tr	4.30 ± 0.04 ^h
B15	40.8 ± 0.2 ^a	tr	0.0209 ± 0.0001 ⁿ	0.95 ± 0.03 ^{fg}	tr	41.8 ± 0.2 ^a
B16	0.40 ± 0.02 ^{cd}	0.11 ± 0.01 ⁿ	0.631 ± 0.003 ^m	n.d.	n.d.	1.15 ± 0.01 ^k

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

Principal component analysis (PCA) is commonly used to reduce the complexity of multivariate data and identify patterns and express data in ways that highlight similarities and differences, and further identify groups of blade samples according to their maturation stage [37,38]. The first seven principal components (PCs) were associated with Eigen values higher than 1 and explained 86.2% of the cumulative variance, with PC1 accounting for 27.4%, PC2 for 18.7%, PC3 for 11.0%, PC4 for 10.1%, PC5 for 7.1%, PC6 for 6.6%, and finally PC7 for 5.2%. PC1 was positively correlated to individual and total tocopherols, whereas it was negatively correlated to quinic and fumaric acid, raffinose, and total sugars. PC2 was positively correlated to trehalose and raffinose and negatively correlated to C20:3n3 and total lipids, γ -tocopherol, malic and citric acid, and sucrose. Finally, PC3 was positively correlated to C24:0, oxalic, citric, and total organic acids and negatively correlated to C20:1. These results indicate a correct application of the PCA, allowing differentiation between the tested maturity stages of blades, as shown in the corresponding scatterplots and loading plots (Figures 2–5). PC1 discriminates maturation stages of cardoon blades, and samples B13 and B14 were distinct from the rest of the samples due to the high contents of α , γ , and total tocopherols and the low contents of fructose, raffinose, total sugars, and quinic acid (Figures 2 and 3). Moreover, PC2 discriminated samples B1, B2, B3, B15, and B16 from the rest of the samples due to the lower contents of malic and citric acid, lipids, and C20:3n3 (Figures 2 and 3). Finally, PC3 discriminated sample B15 due to the highest content of total organic acids and oxalic acid and the lowest content of C16:1 and glucose (Figures 4

and 5). The loading plot of the first two components (Figure 3) also revealed groups of positively correlated variables, namely the upper left quadrant comprising trehalose, fructose, raffinose, quinic, and fumaric acids, and C24:0, the lower left quadrant comprising C13:0, C14:1, and C16:1, sucrose and total sugars, malic and citric acid, and total lipids, the upper right quadrant comprising C20:1, C20:2, oxalic acid, and total organic acids, and the lower right quadrant comprising glucose and tocopherols. Similarly, the loading plot of PC1 and PC3 (Figure 5) revealed groups of positively correlated variables, namely the upper left quadrant comprising fumaric, quinic, and citric acids, C13:0, C24:0, C20:3n3, and lipids, the lower left quadrant comprising raffinose, trehalose, sucrose, fructose, total sugars, and C16:1, the upper right quadrant comprising total organic acids, oxalic acid, and γ -tocopherol, and the lower right quadrant comprising α -tocopherol, total tocopherols, glucose, C20:1, and C20:2.

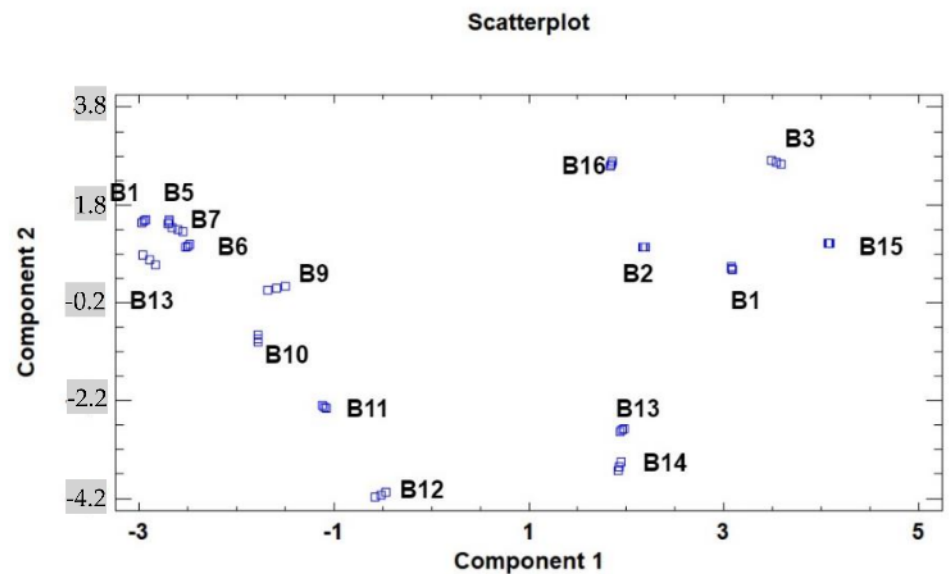


Figure 2. Two-dimensional scatterplot of principal components 1 and 2 for the tested variables at different maturation stages of cardoon blades (samples B1–B16).

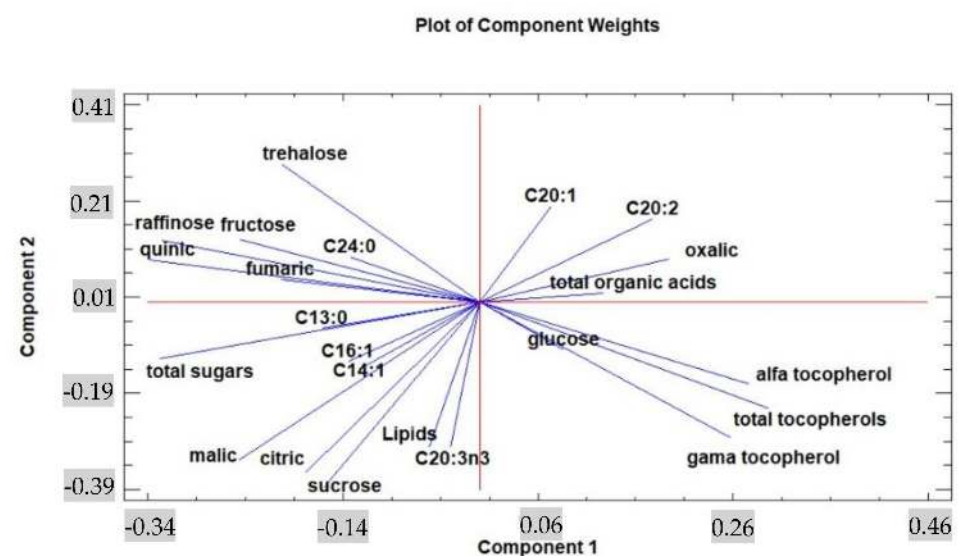


Figure 3. The loading plot of principal components 1 and 2 for the tested variables at different maturation stages of cardoon blades.

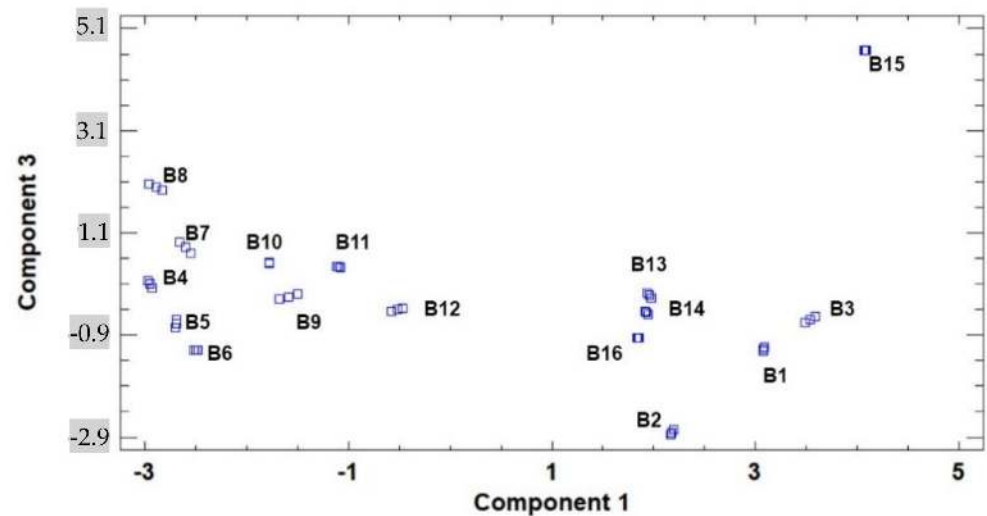


Figure 4. Two-dimensional scatterplot of principal components 1 and 3 for the tested variables at different maturation stages of cardoon blades (samples B1–B16).

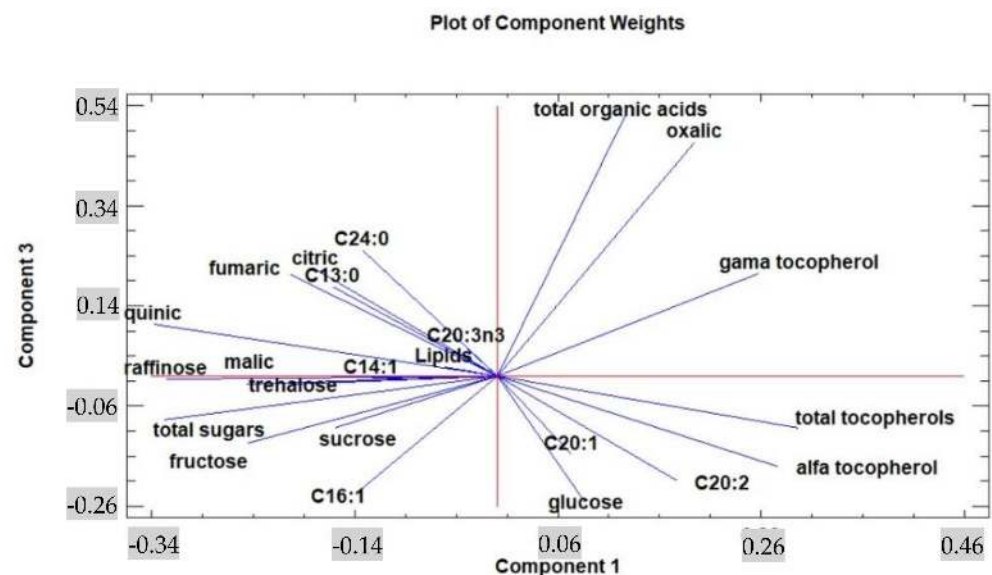


Figure 5. The loading plot of principal components 1 and 3 for the tested variables at different maturation stages of cardoon blades.

4. Conclusions

Cardoon is a species native to the Mediterranean basin and widely consumed due to its rich medicinal and nutritional properties, while it is also used in various industrial applications. It is a crop with a high biomass yield and low maintenance. It also has a high resistance to adverse environmental conditions characteristic of the arid and semi-arid regions of this particular area. Despite presenting all the characteristics that make its cultivation and exploitation viable, the proper use of all of its constituents is extremely important and needs further study. This species annually generates large amounts of by-products, which contribute negatively to the environment and result in wasting plant material rich in compounds of great interest.

Cardoon blades are one of the most frequently discarded or underexploited plant tissues. In this study, the presence of a high variety of molecules of interest was confirmed. Intermediate maturation stages (sample B10, PGS 5/6) had the highest lipid content. Out of the 26 fatty acids identified, palmitic, α -linolenic, and linoleic acids showed the highest relative abundances. In turn, samples with PGS 8–8/9 revealed the highest amounts of

tocopherols and fatty acids, while younger maturation stages (sample B5, PGS 3) had higher concentrations of free sugars. In conclusion, this study provides important information regarding the chemical composition of cardoon blades at different growth stages which could be valorized with targeted harvests for the isolation of specific bioactive compounds for industrial purposes. Therefore, the use and exploitation of all plant tissues of cardoon could be based on the chemical composition of the corresponding stages of development of the plant, making it a significant pillar in the strategy for the economic recovery of the crop and its producing countries.

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