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Chemical Composition of *Cynara cardunculus* L. var. *altilis* Bracts Cultivated in Central Greece: The Impact of Harvesting Time

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Abstract: The present study evaluated the effect of maturity stage on the chemical composition of cardoon bracts. Plant material was collected in Greece at eight different maturation stages (C1–C8) and the chemical composition was analyzed in regard to lipidic fraction and the content in fatty acids, tocopherols, organic acids, and free sugars. Samples of late maturity (C6–C8) revealed the lowest lipidic content, while a total of 29 fatty acids was identified in all the samples, with palmitic, stearic, oleic, and eicosatrienoic acids present in the highest levels depending on harvesting time. Immature (C1) and mature (C8) bracts were more abundant in saturated fatty acids (SFA) than bracts of medium-to-late maturity (C5, C6), where the monounsaturated fatty acids (MUFA) were the prevalent class. The α- and γ-tocopherols were the only identified isoforms of vitamin E, while the highest content was observed in sample C8 (199 μg/100 g dry weight (dw). The detected organic acids were oxalic, quinic, malic, citric, and fumaric acids, while fructose, glucose, sucrose, trehalose, and raffinose were the main detected sugars. The results of the present study allowed us to reveal the effect of maturity stage on cardoon bracts chemical composition and further valorize this byproduct by improving its bioactive compounds content.

Keywords: seasonal variation; chemical composition; free sugars; tocopherols; *Cynara cardunculus* L.; lipidic fraction; fatty acids; organic acids

1. Introduction

Cynara cardunculus L. is a species widely distributed throughout the world, especially in the circum-Mediterranean sea area where it was domesticated for the first time [1]. Belonging to one of the largest families of the plant kingdom, the *Asteraceae* family, this species includes three botanical varieties, namely: the cultivated cardoon (*Cynara cardunculus* var. *altilis* DC), the globe or head artichoke (*Cynara cardunculus* var. *scolymus* (L.) Fiori), and the wild cardoon (*Cynara cardunculus* L. var. *sylvestris* Lamk Fiori). Commonly known as cardoon or artichoke thistle, it is widely used due to its multifaceted properties not only as a food ingredient but also in various industrial applications [2–4].

This species has high nutritional, pharmacological, and industrial value, and, although it has been used since ancient times, it was only in the last decades that cardoon gained attention [5]. In addition to having multiple applications, it is a plant highly resistant to variations in climatic conditions and abiotic stressors, characteristic of the Mediterranean regions [6–8]. Widely consumed in typical Mediterranean

countries as a source of fibers, minerals, and inulin, cardoon is a species that contains a great variety of compounds with important bioactive and nutritional properties. Literature reports refer to the presence of various phenolic compounds, mostly derived from caffeoylquinic and dicaffeoylquinic acids, as well as apigenin and luteolin derivatives, while the presence of sesquiterpenes, lignans, and anthocyanins has also been described [4,9–13].

This actual wealth of compounds with bioactive potential has boosted their exploitation in several sectors of the industry [14]. One of its best-known applications is its use as vegetable rennet for the production of protected designation of origin cheeses (PDO) [3,15]. It is also used for biomass and bioenergy production, as well in the papermaking industry due to its high content of cellulose and hemicellulose [5,16–20]. Its application as a food additive or in nutraceutical and cosmetic products has been also explored [21–23]. There are several studies described in the literature regarding the various industrial applications and biochemical potential associated with cardoon vegetable tissues [5,20,24]. However, about 60% to 85% of the plant material resulting from industrial processes is discarded, increasing the environmental burden and the footprint of the crop and necessitating the channeling of these byproducts in alternative sectors that could increase the added value of the crop. The wasted material consists of bracts, stems, and leaves, which can be a source of important bioactive compounds such as phenolic acids, fibers, minerals, and inulin, which could be used for medicinal and nutraceutical purposes [18,20,21,25–27]. Therefore, the exploitation of these plant tissues can be an important contribution to their economic recovery and reuse, thus reducing waste and sources of environmental contamination and reinforcing the circular economy [5,10,22,28]. In addition, during the whole growth cycle, plants are subjected to variable conditions and cultivation practices. Environmental factors such as the water availability, temperature, light intensity and quality, soil type, and nutritional status, reveal a significant impact on plant metabolism and consequently on the chemical composition of the species throughout the growing season [29–31].

Considering the great amount of waste generated from the cardoon crop, alternative uses of byproducts are essential to increase the added value of this important crop. So far, the studies regarding the influence that the different plant growth stages may have on quality properties such as the chemical composition and the bioactive potential of vegetative tissues are very scarce. Moreover, the valorization of byproducts focuses mostly on biomass and energy production and scarce reports are available regarding the recovering of bioactive compounds from discarded plant parts. Therefore, the aim of this study was to report for the first time the influence that the maturation stage may have on the chemical composition of cardoon bracts collected in central Greece at eight different growth stages, focusing on the lipidic fraction, tocopherols, organic acids, and free sugars composition and content. The presented results could be helpful for the identification of growth stages where the content of specific bioactive contents may increase, as well as for the valorization of such byproducts and the improvement of the overall crop added value.

2. Materials and Methods

2.1. Plant Material

Bracts samples of *Cynara cardunculus* var. *altilis* DC cv. *Bianco Avorio* (Fratelli Ingegnoli Spa, Milano, Italy) were collected during the cultivating period of 2017–2018 at the experimental farm of the University of Thessaly in Velestino, Greece (22.756 E, 39.396 N). Bracts were collected from 15 individual heads for eight harvesting dates according to the principal growth stages (PGS) defined by the Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie (BBCH) scale, comprising stages between PGS 5 and PGS 8/9 [32]. Sample C1 was collected at the end of April (PGS 5), sample C2 at the beginning of May (PGS 5/6), sample C3 at the end of May (PGS 6), sample C4 at the beginning of June (PGS 6/7), sample C5 at the beginning of July (PGS 7), sample C6 at the end of July (PGS 7/8), sample C7 at the beginning of August (PGS 8), and finally, sample C8 was collected at the end of August (PGS 8/9).

The climate conditions and the procedure used for the collection and sampling treatments of plant material were previously described by Mandim et al. [33].

2.2. Chemical Composition Analysis

2.2.1. Fatty Acids

The lipidic fraction of cardoon bracts was extracted through a Soxhlet extraction apparatus with petroleum ether at 120 °C, as recommended by Association of Official Agricultural Chemists (AOAC) procedures [34]. Subsequently, the fat content was subjected to a transesterification process and the fatty acids content was analyzed by Gas-liquid Chromatography (GC), coupled to a Flame Ionization Detector (FID) according to the conditions previously described in Reference [34]. The identification and quantification of fatty acids was performed with the Clarity DataApex 4.0 software (DataApex, Prague, Czech Republic). The identification was based on the comparison of the retention times of the Fatty Acid Methyl Ester (FAME) peaks from samples with commercial standards (reference standard mixture 47885-U; Sigma-Aldrich, St. Louis, MO, USA), and a quantification was made from the area of the peaks. Final results were expressed as relative percentages and in mg of each identified fatty acid per 100 g of dry weight (dw) of plant material.

2.2.2. Tocopherols

The tocopherols content was determined by high-performance liquid chromatography (HPLC, Knauer, Smartline system 1000, Berlin, Germany) coupled to a fluorescence detector (FP-2020, Jasco, Easton, PA, USA) programmed for excitation at 290 nm and emission at 330 nm, according to the procedure described by Barros et al. [35]. The identification and quantification were performed using the Clarity 2.4 software (DataApex, Prague, Czech Republic) and the internal standard (IS) method, through comparison of the retention times and spectra with tocopherols' standards (α-, β-, γ-, and δ-isophorms). Results were expressed in μg per 100 g of dw.

2.2.3. Organic Acids

For organic acids identification, cardoon samples were analyzed by Ultrafast Liquid Chromatography (UPLC, Shimadzu 20A series, Kyoto, Japan) coupled to a Diode Array Detector (UFLC-PDA, Shimadzu Corporation, Kyoto, Japan), according to the chromatographic conditions previously described by Mandim et al. [36]. The identification was performed using the LabSolutions Multi LC-PDA software (Shimadzu Corporation, Kyoto, Japan) and through the comparison of the chromatographic data (retention times and spectra) with commercial standards (oxalic, quinic, malic, ascorbic, citric, and fumaric acids), while their respective calibration curves were used for the quantification based on the area of each peak. Results were presented in g per 100 g of dw.

2.2.4. Free Sugars

The content in free sugars was determined by High-Performance Liquid Chromatography (HPLC, Knauer Smartline 2300, Knauer, Berlin, Germany), coupled to a refractive index detector (RI detector, Knauer Smartline 2300, Knauer, Berlin, Germany), according to the procedure previously described by Dias et al. [37]. The identification and quantification of free sugars were performed using the Clarity 2.4 software (DataApex, Prague, Czech Republic) and through the comparison with commercial standards, namely D-(−)-fructose, D-(+)-sucrose, D-(+)-glucose, D-(+)-trehalose, and D-(+)-raffinose pentahydrate (Sigma-Aldrich, St. Louis, MO, USA).

2.3. Statistical Analysis

The performed assays were carried out in triplicate. The obtained results were presented as mean values ± standard deviation (SD). Means and standard deviations were calculated using Microsoft Excel. SPSS Statistics software (IBM SPSS Statistics for Mac OS, Version 26.0; IBM Corp., Armonk, NY, USA)

was used to determine differences between samples. The results were subject to an analysis of variance (ANOVA), while the Tukey's honest significance test (HSD) test ($p = 0.05$) was used to determine the significant differences among samples.

3. Results and Discussion

3.1. Lipidic Fraction and Fatty Acids Composition

The results related to the lipidic fraction and the fatty acids composition (relative percentage and concentration) are shown in Tables 1 and 2, as well as the proportions of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA), and the PUFA/SFA and n-6/n-3 ratios. Samples collected at late stages of maturity, namely C6, C7, and C8, presented the lowest lipidic content (2.2–2.9 g/100 g dw). In contrast, immature bracts (C2) revealed the highest lipidic levels, being 5.95 times higher than those in the sample of late maturity (C8). Twenty-nine individual fatty acids were identified in cardoon bracts collected at different maturation stages, with palmitic (C16:0, 0.95–44%), stearic (C18:0, 6.64–44.37%), oleic (C18:1n9c, 4.16–29.0%), and eicosatrienoic (C18:2n6c, 2.27–16.852%) acids being present in the highest concentrations. A representative chromatogram of the fatty acids profile is presented in Figure 1 where the retention times and the peaks of the individual detected fatty acids are illustrated. Regarding the effect of maturity stage, palmitic and eicosatrienoic acids were detected in higher levels in immature bracts (samples C1 and C2), while stearic acid revealed higher abundance in bracts of mid-maturity (samples C4, C5, and C6), and oleic acid in bracts of late maturity stages (samples C7 and C8). Saturated fatty acids (SFAs) were the most abundant class of fatty acids in immature bracts (samples C1–C4) and samples of late maturity (samples C7 and C8) due to the high content in palmitic and stearic acids. In turn, monounsaturated fatty acids (MUFAs) were the class with the highest abundance in samples C5 and C6, due to the high content of pentadecanoic acid (C15:1; 31.01–31.2%). Our results also revealed that the tested cardoon bracts did not present an analogous composition and abundance of fatty acids over time, which was also reflected to the recorded PUFA/SFA and n-6/n-3 ratios. The PUFA/SFA ratio was higher than 0.45 in samples C4 and C6, whereas the values of the n-6/n-3 ratio were below 4.0 in all samples, except sample C8. These results verify that the state of maturity influences the composition and abundance of fatty acids in bracts, a finding which is in agreement with previous studies of our team where other cardoon parts were examined [33,38]. In particular, Mandim et al. [33] also reported that lipidic content in cardoon heads decreased with the maturation process and suggested that the differences in the environmental conditions during the growing period could be responsible for the observed differences. Considering that this study was carried out under the same conditions and with the same plant material as in our study, it could be suggested that the environmental factors are also the key drivers for the observed differences in the present study. Similarly, Curt et al. [24], who tested different locations and growing years, highlighted the significant effect of environmental conditions on fatty acid composition of cardoon seeds. To the best of our knowledge, this is the first report that analyzes the influence of the growth cycle on these parameters of chemical composition of bracts, where according to our results, immature bracts (sample C2) presented the highest contents in lipidic components.

Table 1. Lipidic fraction and fatty acids composition of *Cynara cardunculus* bracts in relation to maturity stage (C1–C8).

	C1	C2	C3	C4	C5	C6	C7	C8
Total lipidic fraction (g/100 g dw)								
	4.7 ± 0.1 ^{c}	13.1 ± 0.2 ^{a}	6.1 ± 0.2 ^{b}	4.0 ± 0.1 ^{d}	4.9 ± 0.1 ^{c}	2.9 ± 0.1 ^{e}	2.4 ± 0.2 ^{f}	2.2 ± 0.2 ^{f}
Fatty acids (relative percentage, %)								
C6:0	0.20 ± 0.01 ^{g}	0.26 ± 0.02 ^{f}	0.27 ± 0.03 ^{f}	0.488 ± 0.005 ^{e}	0.56 ± 0.01 ^{d}	0.87 ± 0.01 ^{c}	1.12 ± 0.03 ^{b}	2.28 ± 0.04 ^{a}
C8:0	0.29 ± 0.03 ^{f}	0.33 ± 0.01 ^{e}	0.29 ± 0.03 ^{f}	0.56 ± 0.03 ^{c}	0.615 ± 0.004 ^{b}	0.78 ± 0.02 ^{a}	0.36 n ± 0.01 ^{d}	0.61 ± 0.02 ^{b}
C10:0	0.261 ± 0.002 ^{f}	0.22 ± 0.02 ^{f}	0.7 ± 0.1 ^{c}	0.75 ± 0.06 ^{bc}	0.784 ± 0.001 ^{b}	1.124 ± 0.002 ^{a}	0.52 ± 0.02 ^{e}	0.608 ± 0.001 ^{d}
C11:0	1.066 ± 0.001 ^{b}	0.70 ± 0.02 ^{c}	0.60 ± 0.05 ^{d}	0.62 ± 0.02 ^{d}	0.65 ± 0.01 ^{d}	1.04 ± 0.02 ^{b}	1.03 ± 0.05 ^{b}	1.14 ± 0.05 ^{a}
C12:0	0.65 ± 0.06 ^{e}	1.911 ± 0.004 ^{b}	2.5 ± 0.3 ^{a}	1.46 ± 0.02 ^{c}	1.057 ± 0.004 ^{d}	1.04 ± 0.02 ^{d}	0.62 ± 0.04 ^{e}	0.330 ± 0.003 ^{f}
C13:0	0.095 ± 0.009 ^{a}	0.081 ± 0.001 ^{b}	0.052 ± 0.001 ^{d}	0.092 ± 0.003 ^{a}	0.064 ± 0.001 ^{c}	n.d.	n.d.	n.d.
C14:0	1.9 ± 0.1 ^{d}	2.56 ± 0.01 ^{b}	2.8 ± 0.2 ^{a}	2.63 ± 0.01 ^{ab}	2.19 ± 0.02 ^{c}	1.90 ± 0.02 ^{d}	1.8 ± 0.1 ^{de}	1.7 ± 0.1 ^{e}
C14:1	0.09 ± 0.01 ^{a}	0.064 ± 0.004 ^{b}	0.041 ± 0.001 ^{c}	0.062 ± 0.004 ^{b}	n.d.	n.d.	n.d.	n.d.
C15:0	0.83 ± 0.03 ^{b}	0.70 ± 0.01 ^{d}	0.6 ± 0.1 ^{e}	0.71 ± 0.02 ^{d}	0.866 ± 0.001 ^{b}	1.02 ± 0.01 ^{a}	0.78 ± 0.01 ^{c}	0.74 ± 0.04 ^{cd}
C15:1	0.066 ± 0.003 ^{d}	0.062 ± 0.002 ^{d}	0.043 ± 0.001 ^{d}	0.057 ± 0.003 ^{d}	31.01 ± 0.01 ^{b}	31.2 ± 0.2 ^{a}	0.15 ± 0.01 ^{cd}	0.19 ± 0.01 ^{c}
C16:0	47.3 ± 0.6 ^{a}	47.2 ± 0.1 ^{a}	44 ± 2 ^{b}	0.95 ± 0.01 ^{e}	0.98 ± 0.02 ^{e}	1.27 ± 0.01 ^{e}	36.7 ± 0.4 ^{d}	41 ± 1 ^{c}
C16:1	0.16 ± 0.01 ^{f}	0.257 ± 0.002 ^{e}	0.29 ± 0.03 ^{e}	0.894 ± 0.001 ^{b}	0.79 ± 0.01 ^{c}	1.3 ± 0.1 ^{a}	0.84 ± 0.01 ^{c}	0.49 ± 0.02 ^{e}
C17:0	0.83 ± 0.04 ^{c}	0.699 ± 0.001 ^{d}	0.62 ± 0.03 ^{e}	0.57 ± 0.01 ^{f}	0.545 ± 0.004 ^{f}	0.64 ± 0.02 ^{e}	1.18 ± 0.05 ^{b}	1.3 ± 0.1 ^{a}
C18:0	6.6 ± 0.1 ^{g}	7.58 ± 0.01 ^{f}	8.7 ± 0.5 ^{e}	44.34 ± 0.1 ^{a}	29.80 ± 0.04 ^{b}	24.57 ± 0.01 ^{c}	8.71 ± 0.03 ^{e}	10.4 ± 0.2 ^{d}
C18:1n9c	4.2 ± 0.1 ^{h}	6.90 ± 0.01 ^{g}	14.6 ± 0.3 ^{c}	13.0 ± 0.4 ^{d}	8.14 ± 0.01 ^{f}	10.0 ± 0.1 ^{e}	29.0 ± 0.4 ^{a}	19 ± 1 ^{b}
C18:2n6c	12.59 ± 0.03 ^{b}	16.852 ± 0.001 ^{a}	10.5 ± 0.3 ^{c}	4.96 ± 0.01 ^{d}	2.69 ± 0.03 ^{f}	2.8 ± 0.2 ^{f}	2.3 ± 0.1 ^{g}	3.8 ± 0.2 ^{e}
C18:3n3	5.60 ± 0.02 ^{a}	4.15 ± 0.01 ^{b}	2.7 ± 0.3 ^{c}	0.64 ± 0.01 ^{d}	0.354 ± 0.004 ^{e}	0.51 ± 0.02 ^{de}	0.48 ± 0.02 ^{de}	0.35 ± 0.01 ^{e}
C20:0	4.3 ± 0.1 ^{a}	2.819 ± 0.004 ^{d}	2.43 ± 0.04 ^{e}	0.12 ± 0.01 ^{g}	0.55 ± 0.02 ^{f}	0.63 ± 0.02 ^{f}	3.32 ± 0.02 ^{c}	3.6 ± 0.2 ^{b}
C20:1	0.089 ± 0.002 ^{f}	0.08 ± 0.01 ^{f}	0.52 ± 0.05 ^{d}	0.69 ± 0.01 ^{c}	0.369 ± 0.004 ^{e}	1.06 ± 0.03 ^{a}	1.11 ± 0.05 ^{a}	0.8 ± 0.1 ^{b}
C20:2	0.03 ± 0.01 ^{d}	0.17 ± 0.02 ^{c}	0.35 ± 0.03 ^{a}	0.21 ± 0.01 ^{b}	n.d.	n.d.	n.d.	n.d.
C21:0	0.853 ± 0.002 ^{a}	0.54 ± 0.03 ^{d}	0.17 ± 0.02 ^{e}	0.13 ± 0.02 ^{e}	0.16 ± 0.01 ^{e}	n.d.	0.67 ± 0.05 ^{c}	0.79 ± 0.03 ^{b}
C20:3n6	0.279 ± 0.003 ^{d}	0.16 ± 0.01 ^{e}	n.d.	1.76 ± 0.03 ^{c}	2.078 ± 0.001 ^{a}	1.80 ± 0.02 ^{b}	n.d.	n.d.
C20:3n3	1.6 ± 0.1 ^{c}	1.19 ± 0.03 ^{d}	2.2 ± 0.2 ^{b}	3.4 ± 0.1 ^{a}	0.35 ± 0.01 ^{e}	1.65 ± 0.02 ^{c}	n.d.	n.d.
C22:0	4.58 ± 0.03 ^{a}	3.41 ± 0.04 ^{b}	3.2 ± 0.3 ^{c}	1.9 ± 0.1 ^{f}	1.87 ± 0.02 ^{f}	2.18 ± 0.04 ^{e}	2.9 ± 0.1 ^{d}	4.41 ± 0.03 ^{a}
C22:1	0.59 ± 0.02 ^{d}	0.11 ± 0.01 ^{g}	0.37 ± 0.02 ^{e}	0.80 ± 0.02 ^{c}	1.2 ± 0.1 ^{b}	1.44 ± 0.04 ^{a}	0.405 ± 0.001 ^{e}	0.16 ± 0.01 ^{f}
C20:5n3	0.5 ± 0.1 ^{d}	0.031 ± 0.001 ^{h}	0.20 ± 0.02 ^{g}	1.50 ± 0.05 ^{a}	1.1 ± 0.1 ^{c}	1.20 ± 0.03 ^{b}	0.42 ± 0.01 ^{e}	0.33 ± 0.01 ^{f}
C22:2	0.437 ± 0.001 ^{d}	n.d.	n.d.	16.7 ± 0.5 ^{a}	11.3 ± 0.1 ^{b}	10.052 ± 0.005 ^{c}	n.d.	0.37 ± 0.01 ^{d}
C23:0	1.28 ± 0.02 ^{b}	0.078 ± 0.001 ^{e}	0.74 ± 0.04 ^{d}	n.d.	n.d.	n.d.	1.20 ± 0.01 ^{c}	1.50 ± 0.05 ^{a}
C24:0	2.7135 ± 0.1 ^{c}	0.90 ± 0.04 ^{d}	0.8 ± 0.1 ^{d}	n.d.	n.d.	n.d.	4.4 ± 0.2 ^{a}	4.2 ± 0.2 ^{b}
SFA	73.8 ± 0.1 ^{a}	69.98 ± 0.02 ^{b}	68.3 ± 0.5 ^{c}	55.3 ± 0.1 ^{e}	40.7 ± 0.1 ^{f}	37.05 ± 0.08 ^{g}	65.3 ± 0.4 ^{d}	75 ± 1 ^{a}
MUFA	5.2 ± 0.1 ^{g}	7.47 ± 0.02 ^{f}	15.8 ± 0.3 ^{e}	15.5 ± 0.4 ^{e}	41.5 ± 0.1 ^{b}	44.9 ± 0.1 ^{a}	31.5 ± 0.5 ^{c}	21 ± 1 ^{d}
PUFA	21.1 ± 0.3 ^{c}	22.55 ± 0.01 ^{b}	15.9 ± 0.8 ^{e}	29.2 ± 0.3 ^{a}	17.82 ± 0.03 ^{d}	18.0 ± 0.2 ^{d}	3.2 ± 0.1 ^{f}	4.8 ± 0.2 ^{g}
PUFA/SFA	0.286 ± 0.001 ^{e}	0.322 ± 0.001 ^{d}	0.23 ± 0.01 ^{f}	0.527 ± 0.005 ^{a}	0.4381 ± 0.0001 ^{c}	0.486 ± 0.005 ^{b}	0.048 ± 0.001 ^{h}	0.065 ± 0.004 ^{g}
n-6/n-3	2.32 ± 0.01 ^{e}	3.82 ± 0.01 ^{b}	2.7 ± 0.2 ^{d}	3.1 ± 0.1 ^{c}	3.7 ± 0.1 ^{b}	2.59 ± 0.03 ^{de}	3.1 ± 0.1 ^{c}	6.6 ± 0.5 ^{a}

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; 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; C15:1—pentadecenoic acid; C16:0—palmitic acid; C16:1—palmitoleic acid; C17:0—heptadecanoic acid; C18:0—stearic acid; C18:1n9c—oleic acid; C18:2n6c—linoleic acid; C18:3n3—linolenic acid; C20:0—arachidic acid; C20:1—gadoleic acid; C20:2—eicosadieoic acid; C21:0—heneicosanoic acid; C20:3n6—eicosatrienoic acid; C20:3n3—11,14,17-eicosatrienoic acid; C22:0—behenic acid; C22:1—eicosenoic acid; C20:5n3—eicosapentaenoic acid; C22:2—behenic acid; C23:0—tricosanoic acid; C24:0—lignoceric acid; SFA—saturated fatty acids; MUFA—monounsaturated fatty acids; PUFA—polyunsaturated fatty acids; n-6/n-3: ratio of omega 6/omega 3 fatty acids.

Table 2. Composition of fatty acids (mg/100 g dw) of *Cynara cardunculus* L. bracts in relation to maturity stage (C1–C8; mean ± standard deviation (SD); n = 3).

	C1	C2	C3	C4	C5	C6	C7	C8
				Fatty acids (mg/100 g dw)				
C6:0	9.4 ± 0.5 ^{g}	33 ± 3 ^{b}	17 ± 2 ^{f}	19.5 ± 0.2 ^{e}	27.2 ± 0.4 ^{c}	25.2 ± 0.3 ^{d}	27 ± 1 ^{cd}	50 ± 1 ^{a}
C8:0	13 ± 1 ^{e}	42.9 ± 0.8 ^{a}	18 ± 2 ^{d}	22 ± 1 ^{c}	30.1 ± 0.2 ^{b}	22.6 ± 0.5 ^{c}	8.6 ± 0.2 ^{f}	13.4 ± 0.4 ^{e}
C10:0	12.2 ± 0.1 ^{e}	29 ± 2 ^{d}	44 ± 4 ^{a}	30 ± 3 ^{cd}	38.4 ± 0.1 ^{b}	32.6 ± 0.1 ^{c}	12.5 ± 0.5 ^{e}	13.39 ± 0.03 ^{e}
C11:0	50.13 ± 0.03 ^{b}	92 ± 2 ^{a}	37 ± 3 ^{c}	25 ± 1 ^{e}	32 ± 1 ^{d}	30.2 ± 0.5 ^{d}	25 ± 1 ^{e}	25 ± 1 ^{e}
C12:0	30 ± 3 ^{d}	250.2 ± 0.5 ^{a}	150 ± 16 ^{b}	59 ± 1 ^{c}	51.8 ± 0.2 ^{c}	30.0 ± 0.5 ^{d}	15 ± 1 ^{e}	7.3 ± 0.1 ^{e}
C13:0	4.4 ± 0.4 ^{b}	10.5 ± 0.1 ^{a}	3.2 ± 0.1 ^{d}	3.7 ± 0.1 ^{c}	3 ± 1 ^{d}	n.d.	n.d.	n.d.
C14:0	89 ± 5 ^{d}	335.4 ± 0.9 ^{a}	170 ± 14 ^{b}	105.4 ± 0.4 ^{c}	107 ± 1 ^{c}	55 ± 1 ^{e}	43 ± 2 ^{f}	37 ± 2 ^{f}
C14:1	4.4 ± 0.4 ^{b}	8.3 ± 0.5 ^{a}	2.5 ± 0.1 ^{c}	2.5 ± 0.1 ^{c}	n.d.	n.d.	n.d.	n.d.
C15:0	39 ± 2 ^{c}	92 ± 1 ^{a}	37 ± 4 ^{c}	28 ± 1 ^{d}	42.4 ± 0.1 ^{b}	29.6 ± 0.3 ^{d}	18.6 ± 0.3 ^{e}	16 ± 1 ^{e}
C15:1	3.1 ± 0.1 ^{d}	8.1 ± 0.3 ^{c}	2.6 ± 0.1 ^{d}	2.3 ± 0.1 ^{d}	1519.7 ± 0.5 ^{a}	905 ± 5 ^{b}	3.5 ± 0.2 ^{d}	4.1 ± 0.1 ^{d}
C16:0	2223 ± 30 ^{c}	6183 ± 10 ^{a}	2672 ± 128 ^{b}	38.1 ± 0.5 ^{e}	48 ± 1 ^{e}	36.7 ± 0.2 ^{e}	881 ± 10 ^{d}	901 ± 36 ^{d}
C16:1	7.6 ± 0.3 ^{g}	33.6 ± 0.3 ^{c}	18 ± 2 ^{e}	35.74 ± 0.02 ^{b}	39 ± 1 ^{a}	36 ± 2 ^{b}	20.1 ± 0.2 ^{d}	10.7 ± 0.5 ^{f}
C17:0	39 ± 2 ^{b}	91.5 ± 0.1 ^{a}	38 ± 2 ^{b}	22.5 ± 0.4 ^{e}	26.7 ± 0.2 ^{d}	18.4 ± 0.5 ^{f}	28 ± 1 ^{cd}	29 ± 1 ^{c}
C18:0	312 ± 4 ^{f}	993.0 ± 0.6 ^{c}	531 ± 29 ^{e}	1775 ± 4 ^{a}	1460 ± 2 ^{b}	712.4 ± 0.2 ^{d}	209 ± 1 ^{h}	230 ± 4 ^{g}
C18:1n9c	195 ± 3 ^{f}	903 ± 2 ^{a}	890 ± 17 ^{a}	520 ± 16 ^{c}	398.8 ± 0.4 ^{d}	290 ± 2 ^{e}	697 ± 11 ^{b}	417 ± 24 ^{d}
C18:2n6c	592 ± 2 ^{c}	2208 ± 1 ^{a}	641 ± 20 ^{b}	198.5 ± 0.4 ^{d}	132 ± 2 ^{e}	82 ± 5 ^{f}	54 ± 2 ^{g}	83 ± 5 ^{f}
C18:3n3	263 ± 1 ^{b}	543 ± 1 ^{a}	163 ± 16 ^{c}	25.6 ± 0.3 ^{d}	17.3 ± 0.2 ^{de}	14.9 ± 0.5 ^{e}	11.6 ± 0.4 ^{e}	7.6 ± 0.3 ^{e}
C20:0	202 ± 6 ^{b}	369.3 ± 0.6 ^{a}	148 ± 3 ^{c}	4.9 ± 0.4 ^{g}	27 ± 1 ^{e}	18.4 ± 0.5 ^{f}	80 ± 1 ^{d}	80 ± 4 ^{d}
C20:1	4.2 ± 0.1 ^{e}	11 ± 1 ^{d}	32 ± 3 ^{a}	27.5 ± 0.6 ^{b}	18.1 ± 0.2 ^{c}	31 ± 1 ^{a}	27 ± 1 ^{b}	17 ± 1 ^{c}
C20:2	1.5 ± 0.1 ^{c}	22 ± 2 ^{a}	21 ± 2 ^{a}	8 ± 1 ^{b}	n.d.	n.d.	n.d.	n.d.
C21:0	40 ± 4 ^{b}	70 ± 3 ^{a}	11 ± 1 ^{d}	5 ± 1 ^{e}	7.8 ± 0.3 ^{de}	n.d.	16 ± 1 ^{c}	17.5 ± 0.5 ^{c}
C20:3n6	13 ± 1 ^{e}	21.6 ± 0.7 ^{d}	n.d.	71 ± 1 ^{b}	101.80 ± 0.03 ^{a}	52 ± 1 ^{c}	n.d.	n.d.
C20:3n3	76 ± 1 ^{c}	156 ± 3 ^{a}	131 ± 13 ^{b}	136 ± 3 ^{b}	17.2 ± 0.3 ^{e}	48 ± 1 ^{d}	n.d.	n.d.
C22:0	215 ± 6 ^{b}	447 ± 5 ^{a}	194 ± 19 ^{c}	75 ± 3 ^{e}	92 ± 1 ^{d}	63 ± 1 ^{e}	79 ± 1 ^{e}	97.0 ± 0.5 ^{d}
C22:1	27.7 ± 0.5 ^{d}	14 ± 1 ^{f}	22 ± 1 ^{e}	32 ± 1 ^{c}	58 ± 3 ^{a}	42 ± 1 ^{b}	9.71 ± 0.02 ^{g}	3.6 ± 0.4 ^{h}
C20:5n3	25.2 ± 0.4 ^{d}	3.9 ± 0.1 ^{g}	12 ± 1 ^{e}	60 ± 2 ^{a}	53 ± 4 ^{b}	35 ± 1 ^{c}	9.9 ± 0.2 ^{ef}	7.3 ± 0.2 ^{f}
C22:2	21 ± 2 ^{d}	n.d.	n.d.	669 ± 19 ^{a}	553 ± 7 ^{b}	291.5 ± 0.1 ^{c}	n.d.	8.1 ± 0.2 ^{e}
C23:0	60 ± 1 ^{a}	10.3 ± 0.1 ^{e}	45 ± 2 ^{b}	n.d.	n.d.	n.d.	28.8 ± 0.3 ^{d}	33 ± 1 ^{c}
C24:0	128 ± 6 ^{a}	118 ± 5 ^{b}	50 ± 4 ^{e}	n.d.	n.d.	n.d.	106 ± 4 ^{c}	92 ± 5 ^{d}
SFA	3466 ± 5 ^{c}	9167 ± 3 ^{a}	4164 ± 29 ^{b}	2213 ± 4 ^{d}	1993 ± 3 ^{e}	1074 ± 2 ^{h}	1568 ± 10 ^{g}	1641 ± 31 ^{f}
MUFA	242 ± 4 ^{g}	978 ± 2 ^{c}	967 ± 20 ^{c}	619 ± 16 ^{e}	2034 ± 5 ^{a}	1303 ± 2 ^{b}	756 ± 12 ^{d}	453 ± 26 ^{f}
PUFA	991 ± 1 ^{c}	2955 ± 1 ^{a}	970 ± 49 ^{c}	1167 ± 13 ^{b}	873 ± 1 ^{d}	523 ± 4 ^{e}	76 ± 2 ^{g}	106 ± 4 ^{f}

Results are presented as mean ± standard deviation. Different letters correspond to significant differences ($p < 0.05$). Fatty acids are expressed as mg per 100 g of dw of each fatty acid. dw—dry weight; n.d.—not detected; 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; C15:1—pentadecenoic acid; C16:0—palmitic acid; C16:1—palmitoleic acid; C17:0—heptadecanoic acid; C18:0—stearic acid; C18:1n9c—oleic acid; C18:2n6c—linoleic acid; C18:3n3—linolenic acid; C20:0—arachidic acid; C20:1—gadoleic acid; C20:2—eicosadieoic acid; C21:0—heneicosanoic acid; C20:3n6—eicosatrienoic acid; C20:3n3—11,14,17-eicosatrienoic acid; C22:0—behenic acid; C22:1—eicosenoic acid; C20:5n3—eicosapentaenoic acid; C22:2—behenic acid; C23:0—tricosanoic acid; C24:0—lignoceric acid; SFA—saturated fatty acids; MUFA—monounsaturated fatty acids; PUFA—polyunsaturated fatty acids.

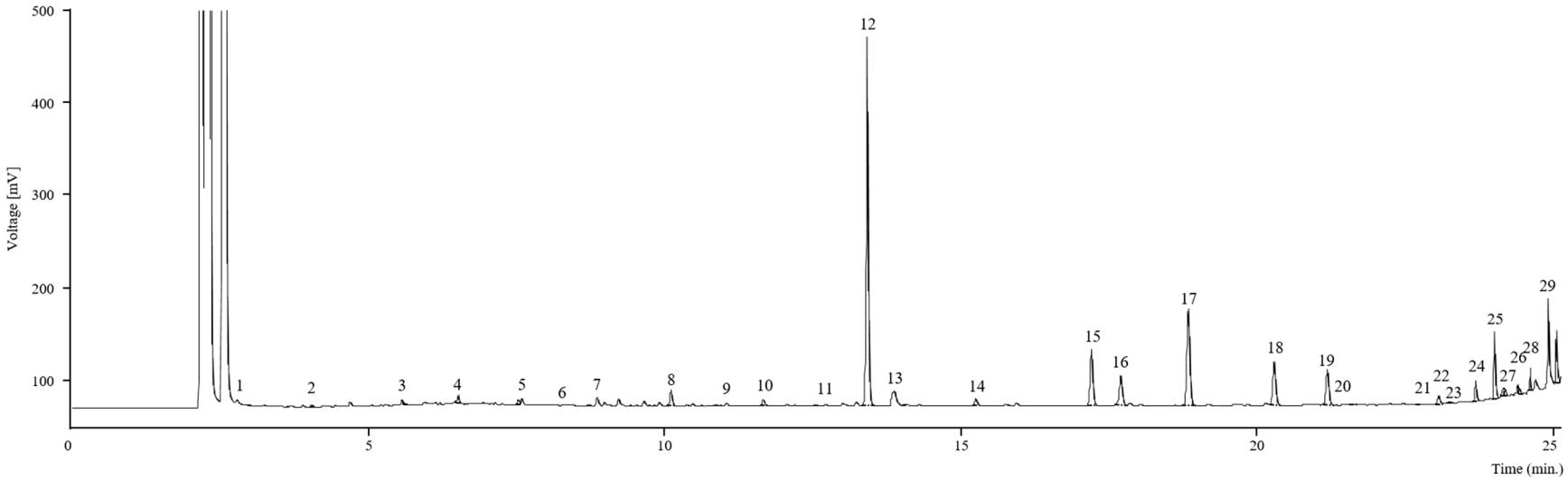

Figure 1. Chromatogram of fatty acids profile of *Cynara cardunculus* bracts (sample C1; collected at the end of April). 1. C6:0—caproic acid; 2. C8:0 caprylic acid; 3. C10:0—capric acid; 4. C11:0—undecanoic acid; 5. C12:0—lauric acid; 6. C13:0—tridecanoic acid; 7. C14:0—myristic acid; 8. C14:1—tetradecanoic acid; 9. C15:0—pentadecanoic acid; 10. C15:1—pentadecenoic acid; 11. C16:0—palmitic acid; 12. C16:1—palmitoleic acid; 13. C17:0—heptadecanoic acid; 14. C18:0—stearic acid; 15. C18:1n9—oleic acid; 16. C18:2n6c—linoleic acid; 17. C18:3n3—alpha-linolenic acid; 18. C20:0—arachidic acid; 19. C20:1—gadoleic acid; 20. C20:2—eicosadieoic acid; 21. C21:0—heneicosanoic acid; 22. C20:3n6—eicosatrienoic acid; 23. C20:3n3—11,14,17-eicosatrienoic acid; 24. C22:0—behenic acid; 25. C22:1—erucic acid; 26. C20:5n3—eicosapentaenoic acid; 27. C22:2—docosadienoic acid; 28. C23:0—tricosanoic acid; 29. C24:0—lignoceric acid.

3.2. Tocopherols, Organic Acids, and Free Sugars Content

In Table 3, the qualitative and quantitative information regarding tocopherols, organic acids, and free sugars identified in the cardoon bracts harvested at different maturation stages are presented. The α- and γ-tocopherols were the only vitamin E isoforms detected in the studied cardoon bracts. Isoform γ-tocopherol was detected in only three maturity stages (samples C2, C4, and C6) in higher concentration than α-tocopherol. The highest abundance of total tocopherols was detected in bracts of late maturity (sample C8; 199 μg/100 g dw), a finding that could be associated with environmental conditions such as solar radiation (light quality and increasing light intensity), as well as the increasing average air temperature. On the contrary, the lowest content of tocopherols was detected in sample C5 (11.7 μg/100 g dw). Due to the fact that tocopherols are antioxidant molecules, they are susceptible to oxidation reactions; thus, they can be strongly influenced by the various environmental conditions to which the plant is subjected throughout its growth cycle [30]. This fact could justify the variations in the content of tocopherols in bracts, as well as the fact that in our previous study [39], phenolic compounds content showed a decrease with increasing maturity, explained by the lignification of bracts tissues [12]. Considering the protective role of tocopherols and polyphenols in the overall antioxidant mechanism of the plant, the observed increase of tocopherols at late maturity could compensate the decreased content of polyphenols and provide defense against abiotic stress. Moreover, although the sample C8 had a higher content in tocopherols and was more efficient to inhibit oxidative hemolysis (OxHLIA), the same was not true for the inhibition of lipid peroxidation (thiobarbituric acid reactive substances; TBARS), where sample C1 revealed a superior antioxidant potential [32]. The same observation was also made in samples of cardoon heads [40], suggesting that other classes of compounds are involved in the antioxidant capacity demonstrated by the analyzed samples. Moreover, it is very common in natural matrices to exhibit variable effectiveness in various antioxidant activity assays, since different compounds and mechanisms are involved in different assays [41,42]. Similarly to our study, the reduced variety of tocopherols found in cardoon bracts has also been reported for other plant parts such as heads and seeds, indicating that α-tocopherol is the main vitamin E isoform detected in the species [30,40,43].

Regarding the organic acids' composition (Table 3), oxalic, quinic, malic, citric, and fumaric acids were the detected compounds. As verified for the other studied parameters, the organic acids composition showed a variation along the maturation process. Bracts collected at the eighth principal growth stage (PSG 8; sample C7) revealed the highest abundance in organic acids (15.6 g/100 g dw), whereas sample C1 (PSG 5) had the lowest abundance (1.96 g/100 g dw). Malic acid was the most relevant organic acid (0.81–1.87 g/100 g dw) at early- to mid-maturation stages (samples C1–C5), whereas in later stages, quinic (samples C6 and C8) and oxalic acids (sample C7) reached the highest concentrations (0.92–4.82 and 9.5 g/100 g dw, respectively). The tested cardoon bracts reveal a similar organic acids profile to that observed for cardoon heads of the same genetic material, with malic acid being present in higher levels in immature heads, while oxalic and quinic acids were more abundant in samples collected at more advanced states of maturity [40].

Table 3. Tocopherols, organic acids, and free sugars of *Cynara cardunculus* L. bracts in relation to maturity stage (C1–C8).

	C1	C2	C3	C4	C5	C6	C7	C8
Tocopherols (μg/100 g dw)								
α-Tocopherol	36.2 ± 0.1 ^{b}	62 ± 2 ^{a}	19.8 ± 0.8 ^{c}	9.4 ± 0.3 ^{e}	11.7 ± 0.5 ^{d}	8.1 ± 0.3 ^{f}	n.d.	199 ± 7 ^{g}
γ-Tocopherol	n.d.	87 ± 3 ^{b}	n.d.	82 ± 3 ^{c}	n.d.	120 ± 2 ^{a}	n.d.	n.d.
Total tocopherols	36.2 ± 0.1 ^{d}	149 ± 1 ^{a}	19.8 ± 0.8 ^{e}	91 ± 5 ^{c}	11.7 ± 0.5 ^{f}	128 ± 2 ^{b}	n.d.	199 ± 7 ^{g}
Organic acids (g/100 g dw)								
Oxalic acid	0.320 ± 0.002 ^{b}	0.328 ± 0.002 ^{b}	0.093 ± 0.002 ^{d}	0.181 ± 0.001 ^{cd}	0.206 ± 0.003 ^{c}	0.129 ± 0.001 ^{cd}	9.5 ± 0.2 ^{a}	0.31 ± 0.01 ^{b}
Quinic acid	0.43 ± 0.01 ^{d}	0.29 ± 0.02 ^{e}	tr	tr	0.056 ± 0.001 ^{f}	0.92 ± 0.01 ^{c}	4.2 ± 0.1 ^{b}	4.82 ± 0.06 ^{a}
Malic acid	0.81 ± 0.02 ^{e}	1.87 ± 0.01 ^{a}	1.42 ± 0.02 ^{d}	1.62 ± 0.02 ^{b}	1.51 ± 0.01 ^{c}	0.40 ± 0.02 ^{f}	0.008 ± 0.001 ^{g}	tr
Citric acid	0.39 ± 0.02 ^{f}	0.55 ± 0.01 ^{d}	0.49 ± 0.02 ^{e}	0.75 ± 0.04 ^{c}	1.15 ± 0.04 ^{b}	0.77 ± 0.03 ^{c}	1.9 ± 0.1 ^{a}	n.d.
Fumaric acid	0.0076 ± 0.0004 ^{a}	0.0049 ± 0.0002 ^{b}	tr	tr	tr	tr	0.0019 ± 0.0001 ^{c}	n.d.
Total organic acids	1.96 ± 0.05 ^{f}	3.042 ± 0.003 ^{c}	2.002 ± 0.004 ^{f}	2.55 ± 0.02 ^{d}	2.92 ± 0.03 ^{c}	2.22 ± 0.04 ^{e}	15.6 ± 0.3 ^{a}	4.95 ± 0.06 ^{b}
Free Sugars (g/100 g dw)								
Fructose	0.41 ± 0.07 ^{e}	1.41 ± 0.09 ^{a}	1.1 ± 0.1 ^{b}	0.91 ± 0.05 ^{c}	0.53 ± 0.08 ^{d}	0.21 ± 0.06 ^{f}	0.14 ± 0.01 ^{f}	0.15 ± 0.02 ^{f}
Glucose	0.144 ± 0.003 ^{d}	0.19 ± 0.01 ^{c}	0.29 ± 0.03 ^{b}	0.29 ± 0.03 ^{b}	0.30 ± 0.01 ^{b}	0.10 ± 0.07 ^{e}	0.27 ± 0.01 ^{b}	0.557 ± 0.004 ^{a}
Sucrose	1.73 ± 0.07 ^{d}	4.97 ± 0.07 ^{a}	2.97 ± 0.03 ^{b}	2.82 ± 0.04 ^{c}	1.3 ± 0.1 ^{e}	0.33 ± 0.07 ^{f}	0.28 ± 0.02 ^{g}	0.12 ± 0.01 ^{h}
Trehalose	0.32 ± 0.06 ^{e}	0.30 ± 0.05 ^{ef}	0.75 ± 0.04 ^{c}	1.16 ± 0.03 ^{a}	0.90 ± 0.02 ^{b}	0.24 ± 0.05 ^{f}	0.34 ± 0.02 ^{e}	0.57 ± 0.02 ^{d}
Raffinose	1.77 ± 0.08 ^{b}	2.13 ± 0.04 ^{a}	1.76 ± 0.04 ^{b}	1.72 ± 0.06 ^{b}	n.d.	n.d.	n.d.	n.d.
Total free sugars	4.4 ± 0.1 ^{c}	9.0 ± 0.2 ^{a}	6.8 ± 0.2 ^{b}	6.9 ± 0.2 ^{b}	3.0 ± 0.2 ^{d}	0.9 ± 0.2 ^{f}	1.03 ± 0.02 ^{f}	1.40 ± 0.03 ^{e}

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. Calibration curves for organic acids: oxalic acid (y = 9106x + 45.973, $R^2 = 0.9901$); Quinic acid (y = 610,607x + 46.061, $R^2 = 0.9995$); Citric acid (y = 1106 x + 45.682, $R^2 = 0.9997$).

The free sugars composition of cardoon bracts is presented in Table 3. A great variation in sugar composition was observed when considering the effect of maturation stage, which suggests that the variation in environmental conditions could be the reason for the observed oscillations. Bracts of early- to mid-maturity stages (samples C1–C6) presented higher concentrations of sucrose and raffinose (0.12–4.97 and 1.72–2.13 g/100 g dw, respectively), whereas in the remaining samples (C7 and C8), the total free sugars content decreased significantly, and trehalose was the sugar present in higher abundance (0.34–0.57 g/100 g dw). Moreover, immature bracts presented higher levels of sugars than the mature ones, a trend that could be associated with the increase of organic acids at late maturity stages. According to Mandim et al. [33], the decrease of free sugars at late maturity stages could be attributed firstly to inulin formation and carbohydrate translocation in other plant parts such as heads, and secondly to the increased needs of osmolytes that help plants to overcome the developing stressful conditions over time, as already reported in other wild species grown under stress conditions [41,44,45]. To the best of our knowledge, this is the first report that analyzes the influence of the growth cycle on these parameters of chemical composition, where according to our results, immature bracts (sample C2) presented the highest contents and total free sugars, while samples with higher grade of maturation (samples C7 and C8) presented the highest content in organic acids and tocopherols.

4. Conclusions

The climatic conditions and the physiological changes to which cardoon plants are subjected throughout their growth cycle have a high impact on their chemical composition. In this work, it was found that the state of maturation has a high influence on the chemical composition of cardoon bracts in regard to lipidic fraction and the content in fatty acids, tocopherols, organic acids, and free sugars. This study is an important contribution to a more complete characterization of the chemical composition of cardoon bracts and reveals how the different phases of growth cycle can influence bioactive compounds content. The obtained results can be used for the sustainable use of bracts through the extraction of compounds with high biochemical value and consequently for the valorization of this species and the increase of the added value of this multifaceted crop.

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