Impact of blanching and frying heating rate/time on the antioxidant capacity and (poly)phenols of cardoon stalks (*Cynara cardunculus* L. var. *altilis* DC)

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A B S T R A C T

This study assessed the influence of blanching and frying heating rate/time on the antioxidant capacity and (poly)phenols of cardoon stalks (*Cynara cardunculus* L. var. *altilis* DC). Blanching (98 °C, 30 s) increased the total native chlorogenic acids content (1.2-fold vs raw cardoon), with no significant changes in DPPH antioxidant capacity, but with a decrease in ABTS antioxidant capacity (0.6-fold). Specifically, total di-caffeoylquinic acids (CQAs) increase (1.6-fold) counterbalanced the losses of 5-CQA (0.8-fold). All frying conditions ($t_{85°C}$ = 5, 12 or 10 min, t_{total} = 15, 15 or 30 min, respectively) decreased the antioxidant capacity (0.5–0.7-fold in DPPH, 0.5–0.9-fold in ABTS) of cardoon, but increased total flavonoid amount (3.6–3.7-fold) that remained at low levels. The Short (15 min) and Intense-heat Frying ($t_{85°C}$ = 5 min) favoured the release of chlorogenic acids, particularly 5-CQA, from the food matrix. However, a longer frying process (30 min) induced an almost complete degradation of di-CQAs. Thus, it is desirable to limit the frying duration. When blanching and frying were combined, a higher thermal degradation of (poly)phenols was observed, but the Short and Intense-heat Frying remained the most suitable. This study highlights the importance of selecting optimal culinary conditions for vegetables that favour a high content on bioactive compounds and, therefore, their potential healthy properties.

1. Introduction

Dietary plant (poly)phenols are natural bioactive compounds with potential nutraceutical properties. In fact, (poly)phenols are credited with a beneficial health role as direct and indirect antioxidants and as modulators of various protein and lipid kinase signaling cascades, which may play a protective role in cancer, proliferative diseases, inflammation, and neurodegeneration (Arfaoui, 2021; Tsao, 2010; Williams et al., 2004). However, these potential health properties are strongly related to the (poly)phenol content of plant foods, which in turn depend on various factors such as food matrix and food processing (Arfaoui, 2021; Murador et al., 2018). Thermal culinary treatments are reported to exert a great impact on the (poly)phenols content and antioxidant capacity of vegetables, which can vary depending on the cooking time and heating degree, and surface area exposed to water, but mainly depend on the food matrix (Murador et al., 2018).

Cultivated cardoon (*Cynara cardunculus* L. var. *altilis* DC) is a vegetable whose cultivation is located in the Mediterranean region, mainly in Spain (Navarra, La Rioja, Aragon and Soria), France, Italy and Southern Portugal where the softer stalks of the plant are consumed as a traditional dish (Pinelli et al., 2007; Ramos et al., 2014). It belongs to *Asteraceae* family which includes two other plants which are the ancestor wild cardoon and the globe artichoke.

Cardoon stalks are usually eaten boiled with water, but also raw in salads (when the stalks are very soft), fried, sautéed, and occasionally baked. Furthermore, an incomplete boiling as occurs with blanching is commonly applied to vegetables before freezing in the agro-food industry. In literature, there are some studies about the content and profile of dietary (poly)phenols in raw cardoon stalks (Juániz et al., 2016, 2017; Petropoulos et al., 2018; Ramos et al., 2014). Among them, Juániz et al. (2016) reported for the very first time the effect of different heat treatments (frying in olive oil, frying in sunflower oil and griddling) on the (poly)phenolic profile and antioxidant capacity of cardoon stalks. Chlorogenic acids were found to be the major dietary (poly)phenols of

cardoon stalks (80–90%), and heat treatment increased the total content of chlorogenic acids (Juániz et al., 2016). Thus, culinary processes can change the content of these bioactive compounds in different ways and, consequently, the antioxidant capacity and potential healthy properties of plant foods. For this reason, detailed studies about the effects of cooking on the content and stability of dietary (poly)phenols are needed to accurately assess the nutritional value of cooked vegetables and to establish the optimal culinary conditions that favour the highest content on bioactive compounds for each vegetable.

Up to our best knowledge, there are no data about the influence of blanching on the total and individual (poly)phenol content and antioxidant capacity of cardoon stalks. In addition, no studies that evaluate the impact of different frying conditions (heating rate and time) after blanching on the content of these bioactive compounds of cardoon have been found. Therefore, the current study aimed to investigate the effects of blanching and frying, applied individually and in combination, on the dietary (poly)phenol content and profile, total flavonoid content, and antioxidant capacity of cardoon stalks.

2. Materials and methods

2.1. Chemicals and reagents

Methanol, ethanol and 98% formic acid of analytical grade, acetonitrile of high performance liquid chromatography (HPLC) grade, Folin-Ciocalteu reagent, sodium carbonate, gallic acid, aluminium chloride hexahydrate and rutin were purchased from Panreac (Barcelona, Spain). Trolox reagent (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,2′-azinobis-(3-ethylbenzothiazonile-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), as well as pure phenolic standard of 5-caffeoylquinic acid (5-CQA), were purchased from Sigma–Aldrich (Steinheim, Germany). In addition, pure phenolic standards of 3,4-dicaffeoylquinic acid (3,4-diCQA), 3,5-dicaffeoylquinic acid (3,5-diCQA) and 4,5-dicaffeoylquinic acid (4,5-diCQA) were acquired from PhytoLab (Vestenbergsgreuth, Germany).

2.2. Preparation of raw and cooked cardoon samples

Cultivated cardoon stalks (*Cynara cardunculus* L. var. *altilis* DC), and extra virgin olive oil were supplied from Spanish local markets. Firstly, two plants of cardoon stalks (around 2.5 Kg each) were washed, and the spiny skin was removed manually. Then, they were cut into rectangular homogeneous pieces (1.5 × 6 cm approx.), manually mixed all together, and divided into 16 portions (300 g each one) in order to prepare the 8 different cardoon samples in duplicate. Two portions were named as raw cardoon and were lyophilized in a freeze dryer Cryodos-80 (Telstar, Terrasa, Spain). The others were cooked as given below.

Blanching: 300 g of cardoon stalks were added to 600 g of boiling water (98 °C) in a stainless-steel pan and cooked for 30 s. The blanching time was measured as soon as the stalks were immersed in water.

Frying: 300 g of cardoon stalks were added over 30 mL of extra virgin olive oil in a non-stick frying pan at 25 °C. Three samples of fried cardoon were prepared under different heating rate and time conditions (Fig. 1). The frying time was measured as soon as the glass-ceramic plate was turned on. In the Short and Soft-heat Frying (SSF, cardoon $t_{85°C}$ = 12 min, t_{total} = 15 min), cardoon temperature increased progressively reaching 85 °C in 12 min (oil temperature at 110 °C), then it was kept at 85-95 °C for 3 min (oil temperature progressively increased up to 135 °C). In the Short and Intense-heat Frying (SIF, cardoon $t_{85°C}$ = 5 min, t_{total} = 15 min), cardoon temperature increased fast reaching 85 °C in 5 min (oil temperature at 110 °C), then it was kept at 85-95 °C for 10 min (oil temperature increased up to 160 °C). In the Long and Soft-heat Frying (LSF, cardoon $t_{85°C}$ = 10 min, t_{total} = 30 min), cardoon temperature reached 85 °C in 10 min (oil temperature at 110 °C), and then it was maintained at 85-95 °C for 20 min (oil temperature increased up to 160 °C).
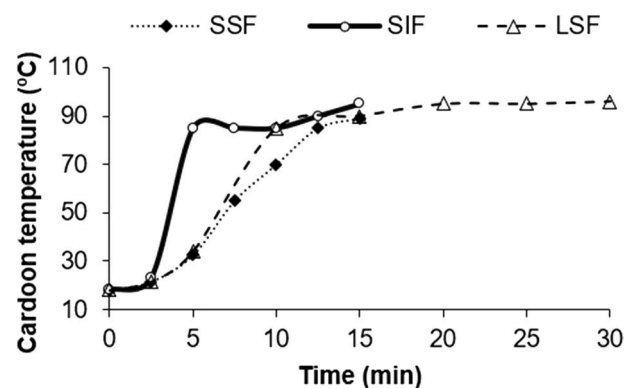

Fig. 1. Frying conditions (time and temperature) applied to cardoon stalks. SSF, Short and Soft-heat Frying ($t_{85°C}$ = 12 min, t_{total} = 15 min); SIF, Short and Intense-heat Frying ($t_{85°C}$ = 5 min, t_{total} = 15 min); LSF, Long and Soft-heat Frying ($t_{85°C}$ = 10 min, t_{total} = 30 min).

Blanching and frying: 300 g of fresh cardoon stalks were submitted to the previous mentioned process of blanching (98 °C, 30 s) and then, they were subjected to each one of the frying conditions previously described, obtaining a total of 3 types of blanched cardoon stalks with a subsequent frying process (i.e., SSF, SIF, LSF).

Temperature and heating time conditions were previously tested to obtain edible cardoon dishes with good sensory characteristics. Water, oil and inner cardoon (approx. 0.5 cm deep) temperature was monitored with a temperature probe. All cardoon samples were lyophilized in a freeze dryer Cryodos-80 (Telstar, Terrasa, Spain), crushed with a chopper (Moulinex, Barcelona, Spain) and stored at −18 °C until further analysis.

2.3. Extraction of (poly)phenols from cardoon samples

(Poly)phenolic compounds from cardoon stalks samples were extracted according to the method described by Juániz et al. (2016). Briefly, 30 mL ethanol/water (80/20 v/v) was added to 2 g of lyophilized cardoon samples. The content was mixed on a mechanical shaker for 1 h at room temperature and then centrifuged at 1610×g for 10 min. The supernatant was collected, and the residue was re-extracted twice by adding 10 mL ethanol/water (80/20 v/v), followed by 1 min vortex, and 5 min centrifugation at 1610×g. The three resulting supernatants were combined and filtered through a Whatman 1 paper. One (poly)phenolic extract was prepared for each duplicate of cardoon sample. Once filtered, extracts were stored at −18 °C until use.

2.4. Antioxidant capacity by DPPH assay

The antioxidant capacity of the different cardoon samples was measured by the 2,2-diphenyl-1-picrylhydrazyl (DPPH•) decolourization assay, following Brand-Williams et al. (1995) procedure with some modifications. Briefly, a freshly prepared DPPH• solution (6.1×10^{-5} M in methanol) was adjusted with methanol to an absorbance of 0.700 (±0.020) at 515 nm in a 3 mL capacity quartz cuvette (1 cm length) at 25 °C (PerkinElmer LAMBDA™ 25 UV/Vis Spectrophotometer, Madrid, Spain). Then, 50 μL of diluted (in distilled water) cardoon sample (poly)phenolic extract were added to 1.95 mL of the DPPH• solution and mixed. The absorbance of the mixture was measured at 515 nm after exactly 18 min. The antioxidant capacity of the extracts against DPPH• was quantified by using a calibration curve of Trolox methanolic solution (a water-soluble vitamin E analogue) at concentrations of 0.05–0.5 mM. Each extract was analysed in triplicate. Results are expressed as the mean of micromoles of Trolox equivalent per gram of cardoon dry matter (μmol Trolox/g dm) ± standard deviation (SD).

2.5. Antioxidant capacity by ABTS assay

The antioxidant capacity of the different cardoon samples was also measured by the ABTS decolourization assay according to the method described by Re et al. (1999) with some modifications (Juaniz et al., 2016). Briefly, a solution consisting of 0.36 mM potassium persulfate and 0.9 mM ABTS was prepared in phosphate buffered saline (PBS) (pH 7.4) and stored in darkness for 12 h to allow the formation of ABTS$^{·+}$ radicals. Then, ABTS$^{·+}$ solution was adjusted with PBS to an absorbance of 0.700 ($\pm$0.020) at 734 nm in a 3 mL capacity plastic cuvette (1 cm length) at 25 °C (PerkinElmer LAMBDA™ 25 UV/Vis Spectrophotometer). Next, 100 µL of each properly diluted (in distilled water) cardoon sample extract was added to 2 mL of ABTS$^{·+}$ solution and homogenized. Then, the absorbance of the mixture was measured at 734 nm after exactly 18 min. The antioxidant capacity of the extracts against ABTS$^{·+}$ radicals was quantified by using a calibration curve of Trolox solution in PBS at concentrations of 0.1–0.5 mM. Each extract was analysed in triplicate. Results are expressed as the mean of micromoles of Trolox equivalent per gram of cardoon dry matter (µmol Trolox/g dm) $\pm$ SD.

2.6. Total phenolic amount by Folin–Ciocalteu assay

Total phenolic compounds in the different cardoon samples were quantified using the Folin–Ciocalteu assay according to the method described by Singleton and Rossi (1965). Briefly, 500 µL of Folin–Ciocalteu reagent were added to a mixture of 100 µL of cardoon sample extract and 7.9 mL of distilled water. After 2 min in agitation, 1.5 mL of 20% v/v sodium carbonate solution were added, and the absorbance of the homogenized mixture was measured at 765 nm (PerkinElmer LAMBDA™ 25 UV/Vis Spectrophotometer, Madrid, Spain) after exactly 90 min of incubation in darkness at 25 °C. Total phenolic amount of each cardoon sample extract was quantified by using a calibration curve of 102–816 µg/mL gallic acid. Each extract was analysed in triplicate. Results are expressed as the mean of milligrams of gallic acid per gram of cardoon dry matter (mg gallic acid/g dm) $\pm$ SD.

2.7. Total flavonoid amount

The estimation of total flavonoid compounds in the different cardoon samples was carried out according to the method described by Lamaison and Carnat (1990) with some modifications (De Santiago et al., 2021). Briefly, 1 mL of diluted (in distilled water) cardoon sample extract was added to 1 mL of 2% aluminium chloride hexahydrate methanolic solution. The mixture was vigorously shaken, and the absorbance was measured at 430 nm (PerkinElmer LAMBDA™ 25 UV/Vis Spectrophotometer, Madrid, Spain) after exactly 10 min of incubation at 25 °C. Total flavonoid amount of each cardoon sample extract was quantified by using a calibration curve of 1–40 µg/mL rutin. Each extract was analysed in triplicate. Results are expressed as the mean of milligrams of rutin equivalent per gram of cardoon dry matter (mg rutin/g dm) $\pm$ SD.

2.8. (Poly)phenolic compounds by HPLC-PDA

(Poly)phenolic compounds in the different cardoon samples were identified and quantified with an analytical HPLC unit model 1200 (Agilent Technologies, Palo Alto, CA, USA) equipped with a binary pump, an auto-sampler injector, and a UV/VIS Photo-Diode-Array detector (PDA). The column used was a C18 5 µm Kinetex 100A (250 × 4.6 mm) (Phenomenex, Maccledfied, UK). Prior to chromatographic separation, each cardoon sample extract was 0.45 µm filtered (Millex-HV Millipore filters). Chromatographic separation was performed following the method described by Juániz et al. (2016) with some modifications. Briefly, 20 µL of each extract was analysed at 37 °C during 57 min using a gradient of distilled water with 0.1% formic acid (mobile phase A) and acetonitrile (mobile phase B) under a constant flow rate of 1.2 mL/min. The gradient profile was: 5–8% B (0–2 min), 8–11% B (2–22 min),

11–15.5% B (22–23 min), 15.5–23% B (23–54 min), 23–80% B (54–55 min), and then return to 5% B in 1 min and maintained the gradient until the end of the analysis (57 min) to re-equilibrate the column. Identification of 5-caffeoylquinic acid (CQA), 3,4-diCQA, 3,5-diCQA and 4,5-diCQA was performed by comparing the retention time and UV spectra with pure standards. In absence of standards, compounds were tentatively identified primarily by means of their UV spectra and available literature data (Juániz et al., 2016). The chromatogram of cardoon stalks submitted to the SIF condition ($t_{85°C}$ = 5 min, t_{total} = 15 min) is shown in Fig. 2 as an example. Quantification was performed by PDA at 325 nm for chlorogenic acids, and they were expressed as 5-CQA equivalents using a 5–200 µg/mL calibration curve. The limit of detection was 7.763 µg/mL and the limit of quantification was 25.855 µg/mL. Chromatograms and spectral data were acquired using the Agilent ChemStation software Rev B.04.02. Each extract was analysed in duplicate. Results are expressed as the mean of milligrams of each (poly) phenol per gram of cardoon dry matter (mg/g dm) $\pm$ SD.

2.9. Statistical analysis

Student *t*-test was used to compare the results between raw and blanched cardoon samples for each parameter. One-way analysis of variance (ANOVA) with a *post hoc* Tukey test was used to compare the results among raw and cooked samples for each parameter. Differences showing *p* value < 0.05 were considered statistically significant. Correlations among tested parameters were performed using Pearson correlation analysis. Analyses were performed using STATA v.12.1 software package.

3. Results and discussion

3.1. Effect of blanching on the antioxidant capacity and (poly)phenols of cardoon stalks

(Poly)phenolic compounds of vegetables are among the major contributors to their antioxidant capacity. Blanching is a cooking method widely used in fruits and vegetables whose main objective is to inactivate the polyphenol oxidase enzymes. These enzymes in the presence of oxygen are responsible for the oxidation of (poly)phenolic compounds to quinones, which are subsequently transformed in dark pigments that lead to browning and loss of nutritional quality of food plants (Doğan et al., 2009; Goncalves et al., 2009). Although blanching process prevents (poly)phenols degradation during storage, the high temperature applied might partially reduce their content. Therefore, it is important to
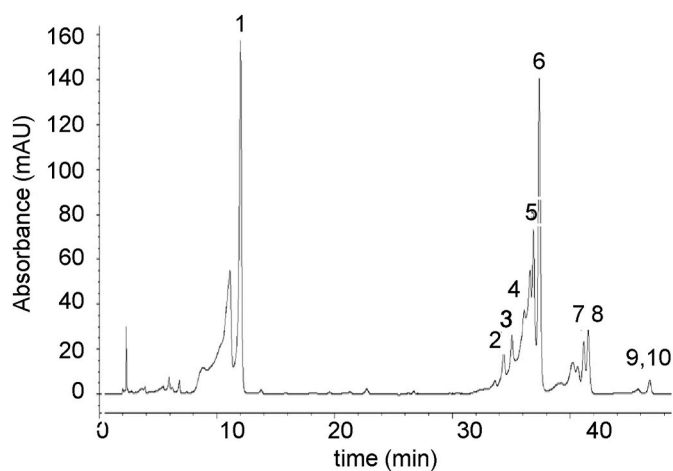

Fig. 2. HPLC-PDA chromatogram of cardoon stalks submitted to SIF ($t_{85°C}$ = 5 min, t_{total} = 15 min) at a wavelength of 325 nm. For peak assignments, see Table 1.

study the impact of blanching on the total and individual (poly)phenolic compounds content and, consequently, on the antioxidant capacity of vegetables. Although there are many studies about the effect of hot water blanching on the (poly)phenolic compounds stability and antioxidant capacity of many *Brassica* species such as cauliflower, broccoli, cabbage and kale (Goncalves et al., 2009; Jaiswal et al., 2012; Managa et al., 2019; Martínez et al., 2020; Volden et al., 2009), and other vegetables such as pea and bean (Wen et al., 2010), and samnamul (a traditional Korean cuisine herb) (Kim et al., 2020), none have been found in cardoon.

The antioxidant capacity of raw cardoon measured by DPPH (39.05 $\pm$ 4.72 µmol Trolox/g dm) (Fig. 3a) and ABTS (54.77 $\pm$ 0.28 µmol Trolox/g dm) (Fig. 3b) was similar to that reported by Juániz et al. (2016). The total phenolic amount quantified in raw cardoon was 8.28 $\pm$ 0.03 mg gallic acid/g dm (Fig. 3c). Regarding (poly)phenolic profile (HPLC analysis), CQAs were the only (poly)phenols in raw cardoon stalks, being 5-CQA, 3,5-diCQA and 1,5-diCQA the most abundant ones (Table 1). Similar results were also reported by Ramos et al. (2014) and Juániz et al. (2016), except for 3,5-diCQA which was not identified by the first one. Also, 1,3- and 1,4-diCQA and one succinyl-diCQA were found in quantities below the limit of quantification. On the contrary, Juániz et al. (2016) did not detect these three diCQAs in raw cardoon stalks but identified and quantified 4,5-diCQA, three succinyl-diCQAs and one disuccinyl-diCQA in low amounts in raw cardoon samples. Petropoulos et al. (2018) reported the presence of 1,3-diCQA and one succinyl-diCQA in raw cardoon leaf midribs and petioles, along with *cis* 3,4-diCQA, *cis* and *trans* 3,5-diCQAs, and 4,5-diCQA. The total chlorogenic acids content in raw cardoon stalks (5.58 $\pm$ 0.13 mg/g dm) (Table 1) is in accordance with that reported by Juániz et al. (2016) (5.22 $\pm$ 0.09 mg/g dm).

Total flavonoids were detected spectrophotometrically in very low amount in cardoon stalks (Fig. 3d), but individual flavonoids were not detected in any raw or cooked cardoon sample by HPLC-DAD, probably due to the extremely low amount of each flavonoid compound. Juániz et al. (2016) reported only trace amounts of flavonoids in both raw and fried samples of cardoon stalks by using UHPLC-DAD-MSn. Ramos et al. (2014) also identified flavonoid compounds in raw cardoon stalks by

using UHPLC-DAD-MSn, but the total flavonoid amount was also considerably lower than the CQA content, which represented the major family of phenolic compounds.

Previous studies assessing the effect of blanching on vegetables report contradictory data on the total phenolic and/or flavonoid content and antioxidant capacity. Some of them reveal that the higher blanching temperature and/or time applied, the greater losses occur in the total phenolic and flavonoid amount and in the antioxidant capacity of vegetables (Goncalves et al., 2009; Jaiswal et al., 2012; Volden et al., 2009). However, an increase in the total phenolic amount at longer processing time has also been reported (Jaiswal et al., 2012). Furthermore, an increase in the total phenolic content and antioxidant capacity of some vegetables after blanching has also been observed (Managa et al., 2019; Tang et al., 2019). In addition, Wen et al. (2010) reported different effects of blanching process (10 min, boiling water) depending on the analysed vegetable. While some blanched vegetables (four-angled bean and French bean) showed higher total phenolic amount, others (snow pea and snap pea) had lower than their corresponding raw ones. Interestingly, Kim et al. (2020) reported that the total phenolic and flavonoid content, and antioxidant activity, of samnamul have dramatic increase up to a maximum, but decrease rapidly when blanching is prolonged at all tested temperatures. The blanching at 98 °C for 30 s showed the highest values among the different blanching temperatures (80, 90, 98 °C) and time (0–10 min) analysed (Kim et al., 2020). Therefore, the aforementioned articles highlight the need to individually optimize the blanching conditions of each vegetable to ensure the intake of its dietary (poly)phenolic compounds.

In the present work, the blanching process (98 °C, 30 s) applied to cardoon stalks did not significantly modify their antioxidant capacity against DPPH radical (Fig. 3a) or the total phenolic amount (Fig. 3c), while the ABTS antioxidant capacity significantly decreased (0.6-fold) (Fig. 3b), and the total flavonoid amount of cardoon stalks significantly increased after blanching when compared to raw, but still remained in very low amount (0.71 $\pm$ 0.06 mg/g dm) (Fig. 3d). These little changes suggest that the lixiviation or transference of water-soluble antioxidant compounds from the cardoon matrix to the processing water could have been minimized by the short time applied (30 s).
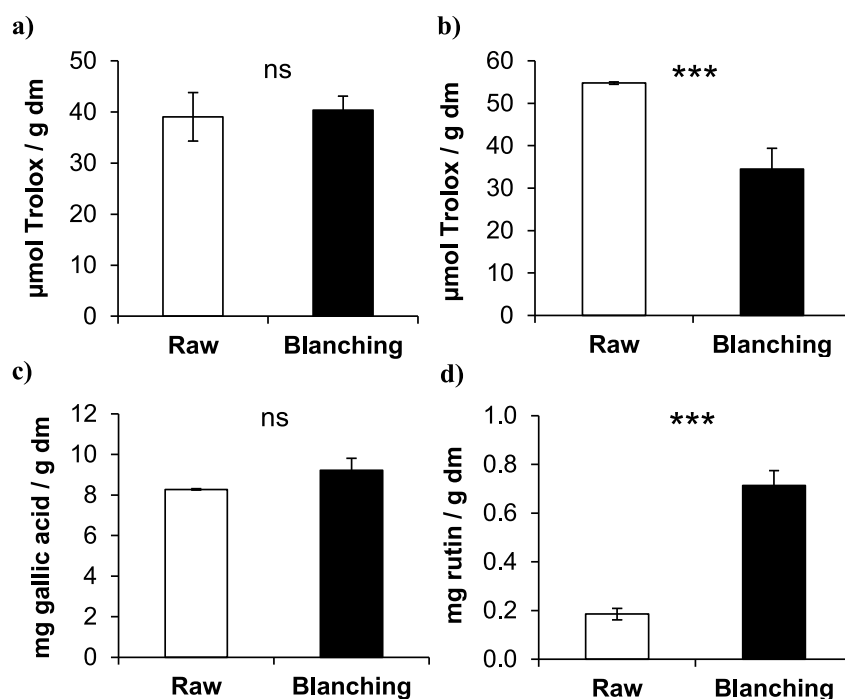

Fig. 3. Antioxidant capacity by (a) DPPH and (b) ABTS assays, and total (c) phenolic and (d) flavonoid amount of raw and blanched (98 °C, 30 s) cardoon stalks. Levels of significance (Student *t*-test) between raw and blanched cardoon stalks samples: ns (not significant) $p \geq 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Table 1

(Poly)phenolic profile of raw, blanched (98 °C, 30 s) and/or fried cardoon stalks. Short and Soft-heat Frying ($t_{85°C}$ = 12 min, t_{total} = 15 min); Short and Intense-heat Frying ($t_{85°C}$ = 5 min, t_{total} = 15 min); Long and Soft-heat Frying ($t_{85°C}$ = 10 min, t_{total} = 30 min). Results are expressed as mean mg (poly)phenol/g dm ± SD. Different letters for each row indicate significant differences (p < 0.05) among samples.

Peak n	Compound	Raw	Without blanching				With blanching		
			Short and Soft-heat Frying	Short and Intense-heat Frying	Long and Soft-heat Frying	Blanching	Short and Soft-heat Frying	Short and Intense-heat Frying	Long and Soft-heat Frying
1	5-CQA	2.93 ± 0.07 f	2.30 ± 0.04 c	3.15 ± 0.01 g	2.63 ± 0.01 e	2.43 ± 0.14 cd	2.09 ± 0.05 b	2.54 ± 0.00 de	1.85 ± 0.07 a
	Total mono-CQAs	**2.93 ± 0.07 f**	**2.30 ± 0.04 c**	**3.15 ± 0.01 g**	**2.63 ± 0.01 e**	**2.43 ± 0.14 cd**	**2.09 ± 0.05 b**	**2.54 ± 0.00 de**	**1.85 ± 0.07 a**
2	1,3-diCQA	tr a	tr a	tr a	nd a	tr a	tr a	tr a	tr a
3	3,4-diCQA	nd a	tr a	tr a	tr a	tr a	tr a	tr a	tr a
4	1,4-diCQA	tr a	0.66 ± 0.03 b	0.68 ± 0.02 b	tr a	1.20 ± 0.05 c	tr a	tr a	tr a
5	3,5-diCQA	1.56 ± 0.04 e	0.96 ± 0.06 bc	1.09 ± 0.14 c	tr a	1.67 ± 0.05 e	0.91 ± 0.04 b	1.02 ± 0.04 bc	tr a
6	1,5-diCQA	1.09 ± 0.02 d	0.92 ± 0.03 c	1.09 ± 0.01 d	tr a	1.23 ± 0.06 e	0.83 ± 0.03 b	0.83 ± 0.01 b	tr a
7	4,5-diCQA	nd a	nd a	tr a	nd a	nd a	tr a	tr a	nd a
	Total di-CQAs	**2.65 ± 0.06 c**	**2.54 ± 0.05 c**	**2.86 ± 0.13 d**	**tr a**	**4.10 ± 0.15 d**	**1.75 ± 0.07 b**	**1.85 ± 0.05 b**	**tr a**
8	Succinyl-diCQA I	nd a	nd a	tr a	nd a	nd a	nd a	nd a	nd a
9	Succinyl-diCQA II	nd a	nd a	nd a	nd a	nd a	nd a	nd a	nd a
10	Succinyl-diCQA III	tr a	nd a	nd a	nd a	nd a	nd a	nd a	nd a
	Total succinyl-di-CQAs	**tr a**	**nd a**	**tr a**	**nd a**	**nd a**	**nd a**	**nd a**	**nd a**
	Total chlorogenic acids	**5.58 ± 0.13 f**	**4.85 ± 0.02 e**	**6.00 ± 0.12 g**	**2.63 ± 0.01 b**	**6.53 ± 0.29 h**	**3.84 ± 0.12 c**	**4.39 ± 0.05 d**	**1.85 ± 0.07 a**

tr, traces (below the limit of quantification); *nd,* not detected.

In addition, the total chlorogenic acids content significantly increased compared to raw cardoon (1.2-fold), but individual chlorogenic acids behaved differently with the blanching treatment (Table 1). Blanched cardoon stalks showed a significant decrease of native 5-CQA content (0.8-fold), while the total amount of di-CQAs significantly increased (1.6-fold) as compared to raw cardoon. More in detail, 1,4-diCQA, which was found in traces in raw cardoon, appeared in much greater amounts in the blanched one (1.20 ± 0.05 mg/g dm), and 1,5-diCQA was also significantly increased (1.1-fold) in blanched cardoon. In addition, 3,4-diCQA became in trace amounts in the blanched cardoon. Generally, (poly)phenols in plants occur both in free forms and covalently bound with macromolecules, or packed in cellular organs or cell wall components (Palermo et al., 2014). The softening effect of the food matrix due to the heat-induced rupture of cell walls and membranes could favour the hydrolysis and further release of bound compounds to the food matrix, and subsequently the lixiviation of lower molecular weight chlorogenic acids, such as 5-CQA, to the blanching water. Furthermore, the higher number of hydroxyl groups in di-CQAs than in mono-CQAs, might favour their interaction with other macromolecules (Monente et al., 2015). Consequently, di-CQAs might be strongly linked to cellular structures, which could explain their increase in blanched cardoon stalks. Moreover, isomerization reactions are also common among chlorogenic acids during heat treatments (Ferracane et al., 2008).

Taking into account all these data, it can be concluded that the blanching temperature and time conditions (98 °C, 30 s) carried out in this study are suitable to preserve the antioxidant capacity (DPPH), as well as to increase the total content of chlorogenic acids, especially di-CQAs, in cardoon stalks, although antioxidant capacity measured by ABTS assay decreased.

3.2. Effect of heating rate/time of frying on the antioxidant capacity and (poly)phenols of cardoon stalks

Cardoon stalks can be eaten raw but are also typically cooked in different ways such as boiled and fried. The effect of the type of oil (olive and sunflower oil) used for frying at certain time and temperature conditions on dietary (poly)phenols from cardoon stalks has been previously study by our group (Juániz et al., 2016). However, since heating can degrade thermo-labile compounds, it is important to know how the frying heating rate and time influence the antioxidant capacity and dietary (poly)phenols of cardoon stalks.

SSF led cardoon stalks to a significant decrease in the antioxidant capacity against DPPH (0.7-fold) and ABTS (0.5-fold) radicals, and the total amount of phenolic compounds (0.8-fold), whereas flavonoids significantly increased (3.6-fold) but remaining at low levels (Fig. 4). In particular, significantly lower levels of 5-CQA (0.7-fold) were observed in SSF cardoon than in the raw one, while total di-CQAs remained at similar concentration due to a counterbalance between the increase of 1,4-diCQA (from traces to 0.66 ± 0.03 mg/g dm) and the decrease of 3,5-diCQA (0.6-fold) and 1,5-diCQA (0.8-fold) (Table 1). SIF induced similar changes in DPPH antioxidant capacity (0.7-fold), total phenolic content (0.8-fold) and total flavonoid content (3.7-fold) in cardoon stalks than SSF, whereas the ABTS antioxidant capacity (0.9-fold) remained similar to that of raw cardoon (Fig. 4). In addition, SIF increased the content of chlorogenic acids, such as 5-CQA and total di-CQAs (Table 1), which might be the result of thermal degradation of cell walls and other cell components leading to the release of bound (poly)phenols to the food matrix (Ramírez-Anaya et al., 2015). Comparing frying procedures with the same time duration, the faster is the heating process the greater might be the release of chlorogenic acids in cardoon stalks, probably due to the higher softening effect observed in SIF cardoon. Therefore, data confirm that the heating rate is a critical parameter that modifies the (poly)phenolic compounds content and the antioxidant capacity of cardoon stalks.

A longer frying process as occur with LSF did not cause significant changes in the antioxidant capacity (DPPH and ABTS) of cardoon stalks in comparison with SIF (Fig. 4a–b, d). However, it caused significant decreases in the total phenolic (0.7-fold) and flavonoid (0.8-fold) amount, but especially in the total chlorogenic acids content (0.4-fold) compared to SIF (Fig. 4c–d, Table 1). This longer frying process induced an almost complete degradation of di-CQAs, and a significant lower level of 5-CQA compared to SIF (0.8-fold) (Table 1). Therefore, when a high heating rate is applied for a period of 30 min, CQAs appear to undergo
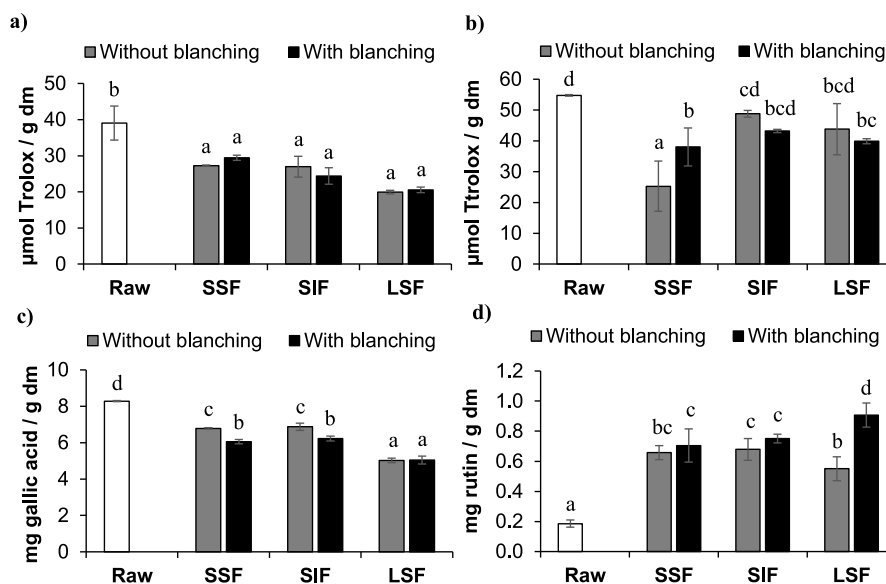
Fig. 4. Antioxidant capacity by (a) DPPH and (b) ABTS assays, and total (c) phenolic and (d) flavonoid amount of raw and fried cardoon stalks subjected or not to a previous blanching (98 °C, 30 s). SSF, Short and Soft-heat Frying ($t_{85°C}$ = 12 min, t_{total} = 15 min); SIF, Short and Intense-heat Frying ($t_{85°C}$ = 5 min, t_{total} = 15 min); LSF, Long and Soft-heat Frying ($t_{85°C}$ = 10 min, t_{total} = 30 min). Different letters indicate significant differences (p < 0.05) among samples.

massive thermal degradation. Consequently, di-CQA compounds are degraded into their corresponding mono-CQAs, such as 5-CQA or other undetectable derivatives, which can also undergo thermal degradation during a longer frying time. Additionally, the formation of other non-phenolic antioxidants during frying such as Maillard reaction products (Ludwig et al., 2014) could balance the loss of antioxidant capacity due to di-CQAs degradation. In fact, cardoon stalks colour became browner with longer frying time. Finally, frying duration seems to be a crucial parameter involved into the thermal degradation of CQAs and consequently, it would be advisable to perform shorter duration frying procedures as carried out in SIF.

3.3. Combined effect of blanching and frying on the antioxidant capacity and (poly)phenols of cardoon stalks

Before consumption, several vegetables such as spinach, artichokes, cabbage, and cardoon stalks are blanched, and subsequently subjected to another cooking procedure.

The impact of blanching (98 °C, 30 s) in combination with frying (SSF, SIF, or LSF) on cardoon stalks antioxidant capacity and total phenolic and flavonoid amount is shown in Fig. 4. The additional application of blanching did not substantially affect the antioxidant capacity on cardoon submitted to any frying condition as compared with their respective unblanched fried samples, except for the SSF that showed significantly higher (1.5-fold) ABTS antioxidant capacity in blanched cardoon (Fig. 4b). The total phenolic amount was slightly lower (0.9-fold) in blanched cardoon submitted to both Short Frying (15 min) conditions (SSF and SIF), than in their respective unblanched fried samples (Fig. 4c). On the contrary, the total flavonoid amount did not significantly change in blanched cardoon samples submitted to the two Short (15 min) Frying processes in comparison with their respective fried samples without blanching, but increased 1.7-fold when submitted to the Long (30 min) Frying (Fig. 4d). In any case, the total flavonoid content in all samples remained very low. This is in agreement with the lack of influence of a further frying to blanched onions, green beans and peas on their flavonoid content (Ewald et al., 1999), and to blanched broccoli on their glucosinolate content (Rungapamestry et al., 2008).

However, the total content of chlorogenic acids was significantly lower in all blanched and fried samples than in their respective unblanched fried samples (between 0.7- and 0.8-fold) (Table 1).

Regarding individual chlorogenic acids, the combination of both heat treatments (blanching and frying) induced the decrease of 5-CQA content in cardoon submitted to any of the three frying conditions compared to their respective fried samples without a previous blanching. In addition, the losses of 5-CQA were significantly higher at a longer frying duration (0.7-fold for LSF) than the Short-heat Frying (0.9- and 0.8-fold for SSF and SIF, respectively). Furthermore, a substantial decrease of 1,4-diCQA to trace amounts in either of the frying conditions performed with a previous blanching was observed, suggesting that the first heat treatment might favour the release of this di-CQA from plant structures, becoming the free compounds more sensitive to a second heating. Also, the content of 1,5-diCQA significantly decreased in blanched cardoon subsequently submitted to frying. In contrast, 3,5-diCQA levels remained similar in blanched cardoon submitted to the two Short frying conditions compared to their unblanched fried samples, which is consistent with the fact that the content of this di-CQA was not altered in blanched cardoon compared to the raw one. In summary, by applying a second heating treatment such as frying after the blanching process, 5-CQA and di-CQAs could be released easier from the blanching-induced damaged food matrix, making them more accessible, and in consequence, more susceptible to thermal degradation, as observed in the lower 5-CQA and di-CQAs contents. Therefore, losses of caffeoylquinic acids in fried blanched cardoon samples could be attributed to blanching-induced lixiviation and further frying-induced thermal degradation.

Blanched cardoon submitted to SIF showed the highest levels of chlorogenic acids among blanched and fried samples (4.39 ± 0.05 mg/g dm), as well as the highest content of 5-CQA (2.54 ± 0.00 mg/g dm) and total di-CQAs (1.85 ± 0.05 mg/g dm) (Table 1). On the contrary, blanched cardoon submitted to LSF showed the lowest levels of total chlorogenic acids (1.85 ± 0.07 mg/g dm), represented mainly by 5-CQA. All these data show that when blanching and frying are combined in cardoon stalks, a Long Frying (30 min) is less advisable than a Short Frying (15 min) because it causes a total degradation of di-CQAs and greater losses of 5-CQA in cardoon stalks. Overall, showing the content of individual and total chlorogenic acids as well as the ABTS antioxidant capacity, the best suitable frying condition was SIF ($t_{85°C}$ = 5 min, t_{total} = 15 min), independently of the application or not of a previous blanching.

Finally, a significant high correlation (r = 0.9008, p < 0.05) between

the total phenolic amount and the DPPH antioxidant capacity of cardoon stalks was found. However, individual or total chlorogenic acids content of cardoon stalks did not significantly (p > 0.05) correlate with the antioxidant capacity measured by DPPH or ABTS radicals. Thus, the antioxidant capacity observed in cardoon stalks does not seem to be due only to chlorogenic acids, but also to other radical-scavenging antioxidants from the food matrix. At high temperatures, Maillard reactions induce the formation of browned compounds, mainly melanoidins. These compounds present antioxidant activity by themselves and because of the presence of (poly)phenolic compounds in their structure (Rufián-Henares and Morales, 2007; López-Galilea et al., 2006). Thus, the antioxidant capacity can be also attributed to the increase of other antioxidants such as melanoidins, which might explain the browning observed in fried cardoon stalks.

4. Conclusion

In summary, a short blanching (98 °C, 30 s) in cardoon stalks allows to increase the total chlorogenic acids content, specifically di-CQAs, with non-significant changes in DPPH antioxidant capacity but a decrease in ABTS antioxidant capacity. Additionally, the frying time has a greater impact on the (poly)phenol content than the frying heating rate. Thus, when cardoon stalks are fried, a rapid temperature increase up to 85-95 °C during the first 5 min is preferable, but it is important to limit the total frying time to the shortest process time (approx. 15 min) in order to favour the release of dietary (poly)phenolic compounds from the food matrix without a subsequent thermal degradation. These frying conditions are recommended regardless the application or not of a previous blanching on cardoon stalks. Finally, when blanching and frying were combined in cardoon stalks, a higher thermal degradation of (poly)phenols was observed. Further studies are needed on the impact of common culinary heat treatments on the profile of dietary (poly) phenolic and other bioactive compounds of other vegetables, with the aim of establishing the optimal processing conditions, as well as to know their bioavailability and bioactivity.

Implications for Gastronomy

Cardoon has gained relevance in Gastronomy during last years due to both the revalorization of traditional recipes in Mediterranean diet and the knowledge of their richness in antioxidants like (poly)phenolic compounds. The knowledge of how thermal culinary processes impact on food bioactive compounds, and therefore on their health related properties is still very limited. Indeed, recent research highlights that during culinary processes some thermolabile antioxidants like vitamins can be degraded, while others like phytochemicals can be released from cellular structures and polymers, counterbalancing the antioxidant capacity. This study explores how culinary techniques applied to cardoon, such as blanching and frying, as well as different time/temperature conditions for frying, impact on (poly)phenols and antioxidant capacity. Thus, knowledge derived from this study allows to investigate what thermal culinary processes are better to preserve the antioxidants of cardoon stalks, as well as to select the optimal frying conditions for enhancing the content of polyphenols and, therefore, the potential healthy properties of cardoon, in a Gastronomy context.

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Author contributions

Estíbaliz Huarte: Methodology, Investigation, Formal Analysis, Writing – Original Draft, Writing – Review & Editing; **Isabel Juániz:** Methodology; **Concepción Cid:** Conceptualization, Supervision, Funding acquisition; **María-Paz de Peña:** Conceptualization, Project Administration, Supervision, Writing – Review & Editing, Funding acquisition.

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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