TITLE: Influence of heat treatment on antioxidant capacity and (poly)phenolic compounds of selected vegetables

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ABSTRACT

The impact of cooking heat treatments (frying in olive oil, frying in sunflower oil and griddled) on the antioxidant capacity and (poly)phenolic compounds of onion, green pepper and cardoon, was evaluated. The main compounds were quercetin and isorhamnetin derivates in onion, quercetin and luteolin derivates in green pepper samples, and chlorogenic acids in cardoon. All heat treatments tended to increase the concentration of phenolic compounds in vegetables suggesting a thermal destruction of cell walls and sub cellular compartments during the cooking process that favor the release of these compounds. This increase, specially that observed for chlorogenic acids, was significantly correlated with an increase in the antioxidant capacity measured by DPPH (r=0.70). Griddled vegetables, because of the higher temperature applied during treatment in comparison with frying processes, showed the highest amounts of phenolic compounds with increments of 57.35%, 25.55% and 203.06% compared to raw onion, pepper and cardoon, respectively.

KEYWORDS: Phenolics; flavonoids; chlorogenic acids; antioxidants; vegetables; heat treatment

1. Introduction

The Mediterranean diet is characterized by the high consumption of fruit and vegetables. The European Union produces a broad range of fruits and vegetables thanks to its varied climatic and topographic conditions and it is one of the main global producers of some vegetables such as tomatoes, carrots and onions. Specifically, 5.7 million tonnes of onions were produced in 2013 in Europe, and Spain was one of the main producer countries with around 21% of total onion production (Eurostat, 2014). This high vegetables production favors their high consumption. Recent data indicate that the consumption of fresh vegetables in Spain in 2014 was 260.96 g/capita/day (MAGRAMA, 2014). This consumption was increased around 31% since 2011 when the intake of fresh vegetables was 179.17 g/capita/day (AECOSAN, 2011). Onion and pepper are two of the most consumed vegetables in Spain, however there are lots of local vegetables as cardoon, chard or borage which have also a high acceptability among population depending on the region (AECOSAN, 2011). In any case, plant foods are the main source of dietary antioxidants, including phenolic compounds. (Poly)phenols rich foods have been reported to exhibit a wide range of biological effects such as protective effects against cardiovascular diseases, neurodegenerative diseases and cancer, probably due to their ability to protect against oxidative damage in cells (Del Rio, Rodriguez-Mateos, Spencer, Tognolini, Borges & Crozier, 2013; Rodriguez-Mateos et al. 2014).

Many dietary vegetables are usually eaten both crude or after cooking in different ways. Culinary processes induce significant changes in foods such as water loss, changes in the total fat content and in the fatty acid profile, degradation of thermolabile compounds, and formation of others due to heat-induced chemical reactions (Miglio, Chiavaro, Visconti, Fogliano & Pellegrini, 2008; Miranda et al. 2010). (Poly)phenolic

compounds can also be affected by thermal processes and, consequently the antioxidant capacity of consumed vegetables too (Ramírez-Anaya, Samaniego-Sánchez, Castañada-Saucedo, Villalón-Mir & de la Derrana, 2015). There are some studies that report the effect of heat treatment on antioxidant activity and (poly)phenolic compounds in vegetables. While boiling is the most investigated cooking method, few studies are about frying process, both deep frying and pan frying (Palermo, Pellegrini & Fogliano, 2014) and as far as we know, only one study was found about the effect of griddling on the antioxidant capacity of vegetables, (Jiménez-Monreal, García-Diz, Martínez-Tomé, Mariscal & Murcia, 2009), but none on the (poly)phenols profile. However, results reported on the effect of heat treatment on the (poly)phenolic compounds are not clear cut. Onion is one of the most studied vegetables, nevertheless both losses and gains in (poly)phenolic compounds after heat treatment are reported in the literature (Price, Bacon & Rhodes, 1997; Crozier, Lean, Morag & Black, 1997; Ewald, Fjelkner-Modig, Johansson, Sjöholm & Akesson, 1999; Lombard, Peffley, Geoffriau, Thompson & Herring, 2005; Rhon, Buchner, Driemel, Rauser & Hroh, 2007; Rodrigues, Pérez-Gregorio, García-Falcón & Simal-Gándara, 2009; Harris, Bruton, Tiwari & Cummins, 2015). The studies found in literature about effect of heat treatment on green pepper are focused on antioxidant activity, but not on (poly)phenolic compound profiles changes (Jiménez-Monreal et al. 2009) and up to our knowledge, this is the first time where the influence of heat treatment on antioxidant capacity and (poly)phenol compounds of cardoon stalks (*Cynara cardunculus* L.) has been studied. Therefore, the aim of this work was to study the impact of three cooking heat treatments (frying in olive oil, frying in sunflower oil and griddled) on the antioxidant capacity and (poly)phenolic compound profiles of onion, green pepper and cardoon, commonly consumed as crude in salads and cooked in several ways in the mediterranean diet.

2. Material and methods

2.1 Chemical and reagents

Yellow onion (*Allium cepa*), sweet Italian green pepper (*Capsicum annuum*), cardoon stalks (*Cynara cardunculus L*), olive oil and sunflower oil were obtained from local stores.

The methanol and ethanol were of analytical grade from Panreac (Barcelona, Spain). The methanol (HPLC grade) was purchased from Panreac (Barcelona, Spain). Trolox (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 (DPPH·), as well as the standards used for identification and quantification of phenolic compounds (quercetin, luteolin, isorhamnetin, 5-caffeoylquinic acid and caffeic acid), were purchased from Sigma-Aldrich (Steinheim, Germany).

2.2 Samples preparation

Chopped vegetables (yellow onion, green pepper, and cardoon stalks) (300 g) were fried with olive or sunflower oils (30 mL) at 115 ºC for 10 minutes in a non-stick frying pan. Then, temperature was decreased to 108 ºC for 5 minutes. Chopped vegetables were also submitted to heating at 150 ºC for 10 minutes and then at 110 ºC for 5 minutes in a non-stick griddle without oil addition. Then, raw and cooked vegetables were lyophilized in a freeze dryer Cryodos-80 (Telstar, Terrasa, Spain), and stored at -18ºC until analysis.

2.3. Vegetables extracts

Vegetables extracts were prepared according to Siddiq et al. (2013) with some modifications. Briefly, thirty mL of ethanol/water (80/20) was added to 2 grams of

lyophilized vegetables. The content was mixed on a mechanical shaker for 1 hour at room temperature and then centrifuged at 4000 rpm for 10 minutes. Supernatant was collected and residues were re-extracted twice using 10 mL of ethanol 80% by vortexing (1 minute) and centrifuged at 4000 rpm for 5 minutes. All three supernatants were combined and frozen at -18ºC for antioxidant capacity and UHPLC-PDA-HR-MS analysis.

2.4. Antioxidant capacity by ABTS assay

The ABTS antioxidant capacity was performed according to the method of Re, Pellegrini, Proteggente, Pannala, Yang & Rice-Evans, (1999). The radicals $ABTS^{\bullet+}$ were generated by the addition of 0.36 mM potassium persulfate to a 0.9 mM ABTS solution prepared in phosphate buffered saline (PBS) (pH 7.4), and the $ABTS^{\bullet+}$solution was stored in darkness for 12 h. The $ABTS^{\bullet+}$ solution was adjusted with PBS to an absorbance of 0.700 (±0.020) at 734 nm in a 3 mL capacity cuvette (1 cm length) at 25 °C (Lambda 25 UV–VIS spectrophotometer, Perkin-Elmer Instruments, Madrid, Spain). An aliquot of 100 μL of each vegetable extract sample properly diluted in demineralized water, was added to 2 mL of ABTS•+ solution. The absorbance was measured spectrophotometrically at 734 nm after exactly 18 min. Calibration was performed with Trolox solution (a water-soluble vitamin E analog), and the antioxidant capacity was expressed as micromoles of Trolox equivalent per gram of dry matter sample (μmol Trolox/g dm).

2.5. Antioxidant capacity by DPPH assay

The antioxidant capacity was also measured using 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) decolorization assay (Brand-Williams, Cuvelier, & Berset, 1995) with some modifications. A 6.1×10^{-5} M $DPPH^{\bullet}$ methanolic solution was prepared immediately before use. The $DPPH^{\bullet}$ solution was adjusted with methanol to an absorbance of 0.700

(±0.020) at 515 nm in a 3 mL capacity cuvette (1 cm length) at 25 °C (Lambda 25 UV–VIS spectrophotometer, Perkin-Elmer Instruments, Madrid, Spain). Vegetable extracts were properly diluted in demineralized water prior to analysis. Samples (50 μL) were added to 1.95 mL of the $DPPH^{\bullet}$ solution. After mixing, the absorbance was measured at 515 nm after exactly 18 min. Calibration was performed with Trolox solution (a water-soluble vitamin E analog). The antioxidant capacity was expressed as micromoles of Trolox equivalent per gram of dry matter sample (μmol Trolox/g dm).

2.6. (Poly)phenolic compounds by UHPLC-PDA-HR-MS

(Poly)phenolic compounds were analysed using an UPLC with a PDA detector scanning from 200-600 nm, equipped with an autosampler cooled at 4 ºC (Dionex Ultimate 3000 RS, Thermo Corporation) and an Exactive™ Orbitrap mass spectrometer fitted with a heated electrospray ionization probe (HESI) (Thermo Fisher Scientific, San José, USA). Chromatographic separation was performed at 40 ºC on a Kinetex 5 μm RP 250 x 4,6 mm reversed phase column (Phenomenex, Macclesfield, UK). Ten microliter of each ethanolic extract was analysed using an 80 min 5 to 50 % gradient of acetonitrile in 0.1 % aqueous formic acid at a constant flow rate of 1 mL/min. After passing the PDA flow cell, the eluate was split and 0.2 mL/min was directed to the mass spectrometer with the HESI operating in negative ionization mode. Analysis was carried out in full-scan (100-800 m/z) and full-scan with In-Source Collision-induced dissociation (CID) (100-800 m/z; CID 25.0 eV). Capillary temperature was 300°C; sheath gas and auxiliary gas were 60 and 20 units/min, respectively; source voltage was 4.0 kV. Identification was achieved by comparing the exact mass and retention time with pure reference standards. In absence of standards, compounds were tentatively identified by comparing the theoretical exact mass of the molecular ion with the experimentally measured accurate mass of the molecular ion. In addition identification was confirmed

by the appearance of typical fragments produced from the molecular ion. Quantification was performed by PDA at 325 nm for caffeic acid glucosides and chlorogenic acids, and at 360 nm for quercetin-, isorhamnetin-, luteolin-, and apigenin derivatives. Typical UPLC-PDA chromatograms of griddled onion, griddled pepper and griddled cardoon are shown in Figure 1. Caffeic acid glucoside was expressed as caffeic acid equivalents by reference to a 0.2–20 μg/mL caffeic acid calibration curve; chlorogenic acids were quantified as 5-Caffeoylquinic acid equivalents by reference to a 0.2–20 μg/mL 5-CQA calibration curve; and quercetin-, isorhamnetin-, luteolin-, and apigenin derivatives were quantified by reference to 0.2-20 μg/mL calibration curves of their respective aglycones.

2.7. Statistical analysis

Each parameter was analysed in triplicate. Results are shown as the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was applied for each parameter. A Tukey test was applied as *a posteriori* test with a level of significance of 95%. Principal Component Analysis (PCA), based on Pearson's correlation matrix, was applied in order to study the effect of heat treatment. All statistical analyses were performed using the STATA v.12.0 software package.

3. Results and discussion

Total of seven flavonoids were identified and quantified in onion samples. All of them were glucosides of quercetin and isorhamnetin. Aglycones of these two flavonoids were not detected. Other minor flavonoids such as kaempferol, luteolin and myricetin derivates, or phenolic acids identified in onion by other authors were not detected in our samples, probably due to differences in onion variety, as well as other factors like

187 cultivar (Sellappan & Akoh,2002; Lanzotti 2006; Rodriguez Galdón, Rodríguez
188 Rodríguez & Díaz Romero, 2008; Lu, Ross, Powers & Rasco, 2011; Simin et al. 2013).
189 The most abundant compounds in onion were three quercetin derivates, one quercetin
190 glucoside and two quercetin diglucosides, which accounted approximately for 90% of
191 total flavonoids in all samples (Table 1). This is similar to the percentage of quercetin
192 diglucosides and monoglucosides (83-93% of the total flavonols content) reported by
193 Lombard et al.(2005) in different raw onions varieties. The total flavonoid content in
194 raw onion obtained by addition of every identified flavonoids by HPLC, was 12.43 mg/
195 100g fresh weight. This concentration is higher than that reported by Rodriguez Galdon
196 et al. (2008) (7.48-9.92 mg/100g fresh weight) but in the lower values of the majority
197 data range described in the literature (12.21 – 62.1 mg/100g fresh weight) (Lombard et
198 al. 2005; Bonaccorsi, Caristi, Gargiulli & Leuzzi, 2005; Sellappan et al. 2002), maybe
199 due to the onion variety taking into account that red onion tends to have higher amounts
200 of flavonoids than yellow ones (Nuutila, Puupponen-Pimiä, Aarni & Oksman-
201 Caldentey, 2003; Lombard et al 2005). In all studies found, quercetin and quercetin
202 derivates were also the main compounds in raw onion.
203 Griddled onion showed the highest amount of flavonoids with an increment of 57.35%
204 compared to raw. Aditionally, the use of olive oil for frying resulted in a higher increase
205 of flavonoids (34.55%) than the use of sunflower oil (15.44%). Quercetin derivates
206 present a higher thermal stability than isorhamnetin derivates, since all phenolic
207 compounds increased with the exception of isorhamnetin glucoside, that dicreased
208 around 90%, remaining quercetin derivates the most abundant after cooking process.
209 However, in literature, both losses and gains in quercetin derivates content after cooking
210 process of onions have been reported depending on the heat treatment conditions.
211 Quercetin derivates did not significantly change when brown-skinned onion was fried

during 5 minutes, whereas at higher frying times (15 minutes) decreased significantly (Price et al. 1997). Lombard et al. (2005) also showed how different heat treatments could affect phenolic content; baking or sauteing increased concentrations of predominant quercetin derivates in 25% and 7%, respectively, compared to raw onions as in the present study, while boiling decreased total flavonoid concentration in 18%. However, other authors reported that frying process induced 21-39% losses of flavonoids in onion (Crozier et al. 1997; Ewald et al. 1999) while others reported no significantly changes in the total levels of quercetin diglucoside and monoglucoside (Rodrigues et al. 2009). In the present study, all compounds tended to increase after cooking process, but molar ratio of quercetin diglucoside/quercetin glucoside decreases with the frying process. In onion fried in olive oil and in sunflower oil, quercetin diglucoside/glucoside had ratios of 0.74 and 0.72, respectively, lower than that of the raw onion (0.83). These results suggest that during frying process, glucosides of the quercetin diglucosides are thermohydrolyzed producing the corresponding monoglucoside as discussed by several authors (Rhon et al. 2007; Rodrigues et al. 2009). However in griddled onion, total onion quercetin diglucosides significantly increased, probably due to the degradation of other quercetin derivates such as quercetin triglucoside, that may be strongly linked to other structures in the food matrix and thus have not been fully extracted. The softening effect of the cooking process of vegetables, in this case mainly griddling in onion, due to heat-induced wall and cells ruptures can also affect (poly)phenolic extractability (Palermo et al., 2014; Harris et al. 2015). Although some authors reported that quercetin monoglucoside can be also deglycosilated by thermohydrolysis to the corresponding aglycon (Rohn et al., 2007, Rodrigues et al. 2009), no quercetin or isorhamnetin aglycons were detected after cooking process in the present work in agreement with Harris et al. (2015), probably

due to both heat degradation and contribution of the formation of Maillard reaction products, like melanoidins (Pérez-Jiménez, Díaz-Rubio, Mesías, Morales & Saura-Calixto, 2014).

The increment of phenolic compounds corresponded with an increase in antioxidant capacity measured by DPPH assay (Figure 2A). DPPH tended to be higher in cooked onions, specially in griddled onion, however this increase was not significant. No data of DPPH in cooked onion were found in literature. On the contrary, raw onion showed the highest antioxidant capacity measured by ABTS (162.10 ± 1.33 μol Trolox / g sample dm) (Figure 2B) and it decreased with the heat treatment, maybe due to the degradation of non phenolic antioxidants. These results disagree with those reported by some authors where no losses or increases in the ABTS antioxidant activity of onion after frying and griddling were found (Jimenez-Monreal et al. 2009; Pellegrini, Miglio, Del Rio, Salvatore, Serafini & Brighenti, 2009).

A total of twelve flavonoids and three phenolic acids were identified in green pepper (Table 2). Flavonoid compounds (quercetin and luteolin derivates) were the main (poly)phenols, accounting for more than 90% of total phenolic compounds in all samples. As well as in onion samples, no aglycone flavonoids were detected. Quercetin rhamnoside and luteolin 7-*O*-(2-apiosyl-6-malonyl) glucoside were the most abundant in all green pepper samples, accounting for around 80% of total phenolic compounds. These results are in agreement with those reported in previous studies in raw green pepper (Marín, Ferreres, Tomás-Barberán & Gil, 2004). In green pepper, also an increase of the total (poly)phenolic compounds after heat treatment was observed. Griddled pepper showed the highest amount of phenolic compounds (0.96 mg (poly)phenolic compounds/ g sample dm). Frying also induced an increase of (poly)phenolic compounds, but the use of olive oil for frying resulted in a lower

increase in flavonoids, and therefore in total (poly)phenolic compounds (48.14%) than with sunflower oil (103.70%). Caffeic acid derivates, as well as luteolin hexoside-pentosides and luteolin glucosides, were only found after heat treatment.

As well as in onion samples, the increase in the identified (poly)phenolic compounds by HPLC also agree with a high antioxidant activity measured by DPPH, with the exception of green pepper fried with olive oil (Figure 2A). By contrast, some authors indicated that radical-scavenging activity (DPPH) differences between raw and stir frying in different pepper samples, including green ones, were not significant (Chuah, Lee, Yamaguchi, Takamura, Yin & Motaba, 2008) or even losses were observed in red pepper (Hwang et al. 2012). These differences, as well as in onion, could be due to different pepper variety and cultivars. On the other hand, and as well as in onion samples, antioxidant activity measured by ABTS assay decreased after heat treatment (Figure 2B) probably due to degradation of thermolabile non-phenolic antioxidants. This decrease was lower in griddled pepper (32.92%) in comparison to green pepper fried with sunflower oil (62.41%) and with olive oil (80.80%). Previous studies also reported losses on scavening capacity (ABTS) in peppers after frying (30-50% losses) and griddling (5-30% losses), but lower because time is shorter (up to 8 min vs 15 min in the present study) (Jimenez-Monreal et al. 2009).

Cardoon is a vegetable from the species of *Cynara cardunculus* L. Some parts of the plant can be edible. In Navarra and other Spanish regions, stalks are consumed fresh in salads (when they are soft) or in typical meals applying frying or griddling. Although several studies about composition and total phenolic compounds of different varieties and parts of *Cynara cardunculus* L. have been found, up to our knowledge only one included stalks, however it is focused on total phenolic compounds by Folin Ciocalteu method and not in the (poly)phenolic profile by HPLC (Velez et al. 2012). In contrast to

the phenolic compounds characteristics of onion and green pepper, the most abundant (poly)phenols of cardoon were chlorogenic acids (four caffeoylquinic acids (CQA), six diccaffeoylquinic acids (diCQA), three succinyldicaffeoylquinic acids (succinyldiCQA) and one disuccinyldicaffeoylquinic acid (dicuccinyldiCQA) (Table 3). These last compounds were reported for the first time in raw cardoon leaves (*C. cardunculus L.*) by Pinelli et al. (2007). In the cardoon stalks of the present study, 5-CQA, 3,5-diCQA and 1,5-diCQA were the most abundant compounds, accounting for 80-90% of total (poly)phenolic compounds detected by HPLC in all samples. Traces of some flavonoids were also detected in cardoon, mainly in griddled one. Up to our knowledge, this is the first time where the influence of heat treatment on antioxidant capacity and (poly)phenol compounds of cardoon stalks (*Cynara cardunculus* L.) has been studied. The total amount of (poly)phenolic compounds identified by HPLC increased after heat treatment. Chlorogenic acids can be found free, but also ionically or covalently attached to other food structures, like melanoidin. DiCQAs, where the addition of a caffeic acid moiety increases the hydroxyl groups, can ionically interact with other macromolecules (Monente, Ludwig, Irigoyen, de Peña & Cid, 2015). The softening effect of the cooking process of vegetable due to heat-induced wall and cells ruptures can affect (poly)phenolic extractability (Palermo et al., 2014), so heat treatment could hydrolyzed and release those compounds from the food matrix. The increment in total (poly)phenolic compounds was higher in griddled cardoon (203.06%) than in cardoon fried in sunflower oil (44.25%) and in cardoon fried in olive oil (25.47%). This could be due to the higher temperature applied during griddled treatment than that applied during frying processes, which might favor the release of these compounds from the food matrix, specially 5CQA which increased dramatically after griddling process probably due to the hydrolysis of diCQAs compounds. On the other hand, the less increment in

312 fried samples may be due to the slight decrease of 5-CQA after frying processes both
313 with olive and sunflower oils, maybe due to isomerization into other CQAs. These
314 results are in agreement with Ferrare et al. (2008) which confirmed that cooking
315 practices caused a marked intramolecular transesterification of caffeoylquinic acid.
316 Highly significant correlations were found between 5-CQA, total CQAs, total diCQAs
317 and total succinyldiCQAs, and antioxidant capacity measured by DPPH assay ($r=0.70$,
318 $p<0.001$), becoming griddled cardoon the most antioxidant sample. Contrary to onion
319 and pepper samples, DPPH values did not increase in cardoon fried with sunflower oil,
320 but as well as in pepper samples, a loss of scavening activity (DPPH) took place in
321 samples fried with olive oil (Figure 2A). On the other hand, ABTS assay showed higher
322 values in cardoon after fried with sunflower oil, and specially after griddled, than raw
323 samples (Figure 2B).
324 Principal Component Analysis (PCA) has been applied to evaluate at a glance the
325 influence of heat treatment on the antioxidant activity and (poly)phenolic compounds of
326 different vegetables. Figure 3 shows the bidimensional representation of all the
327 variables (Figure 3A) and vegetables samples (Figure 3B) according to the two selected
328 Principal Components (PC). PC1 (48.89% of the total variance) was mainly
329 characterized by phenolic compounds, quercetin and isorrhamnetin derivates on the left
330 half graphic, and chlorogenic acids, as well as the scavenging activity measured by
331 DPPH, on the right half graphic. According to this, cardoon samples which are
332 characterized by the presence of chlorogenic acids and higher antioxidant capacity than
333 onion and pepper samples, are found in the right part of the graphic. In the left half part,
334 there are vegetable samples characterized by flavonoids, specifically quercetin and
335 isorhamnetin derivates in onion and in lesser extent in green pepper. PC2, which
336 explained 29.64% of the total variance, is characterized by luteolin and caffeic acid

337 derivates. In fact, luteolin derivates are the characteristic (poly)phenolic compounds of
338 green pepper samples. Despite each vegetable is grouped according to its characteristic
339 (poly)phenolic compounds, it can be observed how cooked vegetables, particularly
340 griddled samples, tend to increase the concentration of phenolic compounds, being in
341 the extremes positions. This increase, specially that observed for chlorogenic acids
342 (CQAs, diCQAs and succinyldiCQAs), was significantly correlated with an increase in
343 the antioxidant capacity measured by DPPH ($r=0.70$, $p<0.001$). In fact, griddled
344 cardoon is the vegetable with both, the highest amount of phenolic compounds and the
345 highest scavenging activity (DPPH). The higher temperature during griddling than that
346 applied during frying might favor the release of all these polyphenolic compounds from
347 the food matrix. However, scavenging capacity measured by ABTS remained
348 unexplained by the identified (poly)phenolic compounds, but it might be related to other
349 nonphenolic antioxidants present in vegetables.

350 **4. Conclusions**

351 All heat treatments tended to increase the (poly)phenols content in vegetables
352 suggesting a thermal destruction of cell walls and sub cellular compartments during the
353 cooking process that favor the release of these compounds. Griddled vegetables,
354 because of the higher temperature applied during treatment in comparation with frying
355 processes, showed the highest amounts of phenolic compounds with increments of
356 57.35%, 25.55% and 203.06% compared to raw onion, pepper and cardoon,
357 respectively. Although, up to now, there is no direct relationship between chemical
358 extractability and bioaccesibility, the rupture of the plant structures could facilitate the
359 action of gastrointestinal enzymes and might increase the bioavailability of
360 (poly)phenolic compounds and, consequently, their health benefits. However, further
361 research is required to investigate if the increase in (poly)phenolic compounds, caused

362 by heat treatment also increases the bioavailability of these compounds after
363 consumption.

364

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374 **References**

375 AECOSAN (Agencia Española de Consumo, Seguridad Alimentaria y Nutrición).
376 (2011). ENIDE: Encuesta nacional de ingesta dietética (2009-2010). Resultados
377 sobre datos de consumo. URL
378 http://www.aesan.mspsi.gob.es/AESAN/docs/docs/evaluacion_riesgos/datos_consu
379 mo/ENIDE.pdf. Accessed 04.08.15

380 Bonaccorsi, P., Caristi, C., Gargiulli, C., & Leuzzi, U. (2005). Flavonol glucoside
381 profile of southern italian red onion (*Allium cepa* L.). *Journal of Agricultural and*
382 *Food Chemistry*, 53(7), 2733-40.

383 Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method
384 to evaluate antioxidant activity. *LWT - Food Science and Technology*, *28*(1), 25-30.

385 Chuah, A. M., Lee, Y., Yamaguchi, T., Takamura, H., Yin, L., & Matoba, T. (2008).
386 Effect of cooking on the antioxidant properties of coloured peppers. *Food*
387 *Chemistry, 111*(1), 20-28.

388 Crozier, A.; Lean, M. E. J; Morag S. Mc.D. & Black C. (1997). Quantitative analysis of
389 the flavonoid content of commercial tomatoes, onions, lettuce, and celery. *Journal*
390 *of Agricultural and Food Chemistry,* 45(3), 590–595.

391 Del Rio, D., Rodriguez-Mateos, A., Spencer, J. P.E., Tognolini, M., Borges, G. &
392 Crozier, A. (2013). Dietary (poly)phenolics in human health: Structures,
393 bioavailability, and evidence of protective effects against chronic diseases.
394 *Antioxidants & Redox Signaling, 18*(14), 1818-1892.

395 Eurostat. (2014). Statistical books. Agriculture, forestry and fishery statistics 2014
396 edition. URL http://ec.europa.eu/eurostat/documents/3217494/6639628/KS-FK-14-
397 001-EN-N.pdf/8d6e9dbe-de89-49f5-8182-f340a320c4bd Accessed 04.08.15

398 Ewald, C., Fjelkner-Modig, S., Johansson, K., Sjöholm, I., & Åkesson, B. (1999). Effect
399 of processing on major flavonoids in processed onions, green beans, and peas.
400 *Food Chemistry, 64*(2), 231-235

401 Ferracane, R., Pellegrini, N., Visconti, A., Graziani, G., Chiavaro, E., Miglio, C., &
402 Fogliano, V. (2008). Effects of different cooking methods on antioxidant profile,
403 antioxidant capacity, and physical characteristics of artichoke. Journal of
404 Agricultural and Food Chemistry, 56(18):8601-8608.

405 Harris, S., Brunton, N., Tiwari, U., & Cummins, E. (2015). Human exposure modelling
406 of quercetin in onions (allium cepa L.) following thermal processing. Food
407 Chemistry, 187, 135-139.

408 Hwang, I. G., Shin, Y. J., Lee, S., Lee, J., & Yoo, S. M. (2012). Effects of different
409 cooking methods on the antioxidant properties of red pepper (capsicum annuum
410 L.). *Preventive Nutrition and Food Science*, 17(4), 286-292.

411 Jiménez-Monreal, A. M., García-Diz, L., Martínez-Tomé, M., Mariscal, M. & Murcia,
412 M. A. (2009). Influence of cooking methods on antioxidant activity of vegetables.
413 *Journal of Food Science*, *74*(3), H97-H103.

414 Lanzotti, V. (2006). The analysis of onion and garlic. *Journal of Chromatography A*,
415 *1112*(1–2), 3-22.

416 Lombard, K., Peffley, E., Geoffriau, E., Thompson, L., & Herring, A. (2005). Quercetin
417 in onion (*Allium cepa* L.) after heat-treatment simulating home preparation.
418 *Journal of Food Composition and Analysis*, *18*(6), 571-581.

419 Lu, X., Ross, C.F., Powers, J.R. &. Rasco, B.A. (2011). Determination of quercetins in
420 onion (*Allium cepa*) using infrared spectroscopy. *Journal of Agricultural and Food*
421 *Chemistry*. 59(12):6376-82.

422 MAGRAMA (Ministry of Agriculture, Food and Environment). (2014). La
423 alimentación mes a mes en España , Agosto 2014 URL
424 http://publicacionesoficiales.boe.es/detail.php?id=630928014-0008. Accessed
425 04.08.15

426 Marín, A., Ferreres, F., Tomás-Barberán, F. A. & Gil, M. I. (2004). Characterization
427 and quantitation of antioxidant constituents of sweet pepper (*Capsicum annuum*
428 L.). *Journal of Agricultural and Food Chemistry,* 52(12):3861-9.

429 Miglio C., Chiavaro E., Visconti A., Fogliano V.& Pellegrini N. (2008). Effects of
430 different cooking methods on nutritional and physicochemical characteristics of
431 selected vegetables. *Journal of Agricultural and Food Chemistry*, *56*(1), 139-147.

432 Miranda, J. M., Martínez, B., Pérez, B., Antón, X., Vázquez, B. I., Fente, C. A., Franco,
433 C.M., Rodríguez, J.L & Cepeda, A. (2010). The effects of industrial pre-frying and
434 domestic cooking methods on the nutritional compositions and fatty acid profiles of
435 two different frozen breaded foods. *LWT - Food Science and Technology, 43*(8),
436 1271-1276.

437 Monente, C., Ludwig, I. A., Irigoyen, A., De Peña, M.P. & Cid, C. (2015). Assessment
438 of total (free and bound) phenolic compounds in spent coffee extracts. *Journal of*
439 *Agricultural and Food Chemistry,* 63 (17), 4327–4334

440 Nuutila, A. M., Puupponen-Pimiä, R., Aarni, M. & Oksman-Caldentey, K. (2003).
441 Comparison of antioxidant activities of onion and garlic extracts by inhibition of
442 lipid peroxidation and radical scavenging activity. *Food Chemistry, 81*(4), 485-493.

443 Palermo, M., Pellegrini, N. & Fogliano, V. (2014). The effect of cooking on the
444 phytochemical content of vegetables. *Journal of the Science of Food and*
445 *Agriculture, 94*(6), 1057-1070.

446 Pellegrini, N., Miglio, C., Del Rio, D., Salvatore, S., Serafini, M. & Brighenti, F.
447 (2009). Effect of domestic cooking methods on the total antioxidant capacity of
448 vegetables. *International Journal of Food Sciences and Nutrition, 60*(s2), 12-22.

449 Pérez-Jiménez, J., Díaz-Rubio, M. E., Mesías, M., Morales, F. J. & Saura-Calixto, F.
450 (2014). Evidence for the formation of maillardized insoluble dietary fiber in bread:
451 A specific kind of dietary fiber in thermally processed food. *Food Research*
452 *International, 55*(0), 391-396.

453 Pinelli, P., Agostini, F., Comino, C., Lanteri, S., Portis, E. & Romani, A. (2007).
454 Simultaneous quantification of caffeoyl esters and flavonoids in wild and cultivated
455 cardoon leaves. *Food Chemistry, 105*(4), 1695-1701.

456 Price, K. R., Bacon, J. R. &. Rhodes, M. J. C. (1997). Effect of storage and domestic
457 processing on the content and composition of flavonol glucosides in onion (allium
458 cepa). *Journal of Agricultural and Food Chemistry*, 45 (3), pp 938–942.

459 Ramírez-Anaya, J. d. P., Samaniego-Sánchez, C., Castañeda-Saucedo, M. C., Villalón-
460 Mir, M. & de la Serrana, H. L. (2015). Phenols and the antioxidant capacity of
461 mediterranean vegetables prepared with extra virgin olive oil using different
462 domestic cooking techniques. *Food Chemistry, 188*(0), 430-438.

463 Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. & Rice-Evans, C. (1999).
464 Antioxidant activity applying an improved ABTS radical cation decolorization
465 assay. *Free Radical Biology and Medicine, 26*(9–10), 1231-1237.

Rodrigues, A. S., Pérez-Gregorio, M. R., García-Falcón, M. S. & Simal-Gándara, J. (2009). Effect of curing and cooking on flavonols and anthocyanins in traditional varieties of onion bulbs. *Food Research International, 42*(9), 1331-1336.

Rodríguez Galdón, B., Rodríguez Rodríguez, E. M. & Díaz Romero, C. (2008). Flavonoids in onion cultivars (*Allium cepa* L.). *Journal of Food Science, 73*(8), C599-C605.

Rodríguez-Mateos, A., Vauzour, D., Krueger, C. G., Shanmuganayagam, D., Reed, J., Calani, L. et al. (2014). Bioavailability, bioactivity and impact on health of dietary flavonoids and related compounds: An update. *Archives of Toxicology*, 88(10), 1803-1853.

Rohn, S., Buchner, N., Driemel, G., Rauser, M. & Kroh. L. W. (2007). Thermal degradation of onion quercetin glucosides under roasting conditions. *Journal of Agricultural and Food Chemistry.* 55 (4), 1568–1573.

Sellappan, S. & Akoh, CC. (2002). Flavonoids and antioxidant capacity of georgia-grown vidalia onions. *Journal of Agricultural and Food Chemistry*. 50(19):5338-42.

Siddiq, M., Roidoung, S., Sogi, D. S. & Dolan, K. D. (2013). Total phenolics, antioxidant properties and quality of fresh-cut onions (*Allium cepa* L.) treated with mild-heat. *Food Chemistry, 136*(2), 803-806.

Simin, N., Orcic, D., Cetojevic-Simin, D., Mimica-Dukic, N., Anackov, G., Beara, I., .Mitic-Culafic, D. & Bozin, B. (2013). Phenolic profile, antioxidant, anti-

487 inflammatory and cytotoxic activities of small yellow onion (*Allium flavum* L.
488 subsp. *flavum*, Alliaceae). *LWT - Food Science and Technology, 54*(1), 139-146.

489 Velez, Z., Campinho, M. A., Guerra, Â. R., García, L., Ramos, P., Guerreiro, O.,
490 Felicio, L., Schmitt, F. & Duarte, M. (2012). Biological characterization of *cynara*
491 *cardunculus* L. methanolic extracts: Antioxidant, anti-proliferative, anti-migratory
492 and anti-angiogenic activities. *Agriculture, 2*, 472-492.

Table 1. (Poly)phenolic compounds in raw and cooked onions (fried in olive oil, fried in sunflower oil and griddled). Results are expressed as mean ± standard deviation (mg/g sample dm).

Peak no.	Compound	Raw	Fried in olive oil	Fried in sunflower oil	Griddled
1	Quercetin triglucoside	0.01 ± 0.00 ^{a}	0.01 ± 0.00 ^{a}	0.01 ± 0.00 ^{a}	0.01 ± 0.00 ^{a}
2	Quercetin diglucoside I	0.22 ± 0.00 ^{a}	0.30 ± 0.01 ^{a}	0.24 ± 0.03 ^{a}	0.35 ± 0.06 ^{a}
3	Quercetin diglucoside II	0.35 ± 0.00 ^{a}	0.42 ± 0.01 ^{b}	0.38 ± 0.02 ^{ab}	0.56 ± 0.03 ^{c}
5	Quercetin glucoside I	0.04 ± 0.00 ^{b}	0.02 ± 0.00 ^{a}	0.02 ± 0.00 ^{a}	0.03 ± 0.00 ^{ab}
6	Quercetin glucoside II	0.64 ± 0.01 ^{a}	0.95 ± 0.01 ^{c}	0.83 ± 0.00 ^{b}	1.02 ± 0.04 ^{c}
	Total Quercetin derivates	**1.26 ± 0.01** ^{a}	**1.71 ± 0.03** ^{bc}	**1.48 ± 0.05** ^{ab}	**1.96 ± 0.12** ^{c}
4	Isorhamnetin diglucoside	0.02 ± 0.00 ^{a}	0.12 ± 0.02 ^{bc}	0.09 ± 0.02 ^{b}	0.17 ± 0.02 ^{c}
7	Isorhamnetin glucoside	0.08 ± 0.00 ^{a}	0.01 ± 0.00 ^{a}	0.01 ± 0.00 ^{a}	0.01 ± 0.00 ^{a}
	Total Isorhamnetin derivates	**0.10 ± 0.01** ^{a}	**0.13 ± 0.02** ^{ab}	**0.10 ± 0.02** ^{a}	**0.18 ± 0.01** ^{b}
	Total Flavonoids	**1.36 ± 0.01** ^{a}	**1.83 ± 0.05** ^{bc}	**1.57 ± 0.07** ^{ab}	**2.14 ± 0.14** ^{c}

Different letters for each row indicate significant differences ($p \leq 0.05$) among samples

Table 2. (Poly)phenolic compounds in raw and cooked green pepper (fried in olive oil, fried in sunflower oil and griddled). Results are expressed as mean ± standard deviation (mg/g sample dm).

Peak no.	Compound	Raw	Fried in olive oil	Fried in sunflower oil	Griddled
4	Quercetin rhamnoside glucoside I	nd^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}
11	Quercetin rhamnoside glucoside II	nd^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.02 ± 0.00^{b}
6	Quercetin 3-sambubioside-7-rhamnoside	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.02 ± 0.00^{a}
14	Quercetin rhamnoside	0.17 ± 0.00^{a}	0.19 ± 0.02^{ab}	0.24 ± 0.01^{b}	0.42 ± 0.01^{c}
13	Quercetin glucoside	0.01 ± 0.00^{a}	0.02 ± 0.00^{a}	0.04 ± 0.00^{ab}	0.06 ± 0.00^{b}
	Total Quercetin derivates	**0.18 ± 0.41^{a}**	**0.23 ± 0.02^{a}**	**0.32 ± 0.02^{b}**	**0.53 ± 0.01^{c}**
9	Luteolin glucoside I	nd^{a}	tr^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}
10	Luteolin glucoside II	nd^{a}	tr^{a}	0.01 ± 0.00^{a}	0.02 ± 0.00^{a}
5	Luteolin hexoside pentoside I	nd^{a}	tr^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}
7	Luteolin hexoside pentoside II	nd^{a}	tr^{a}	tr^{a}	tr^{a}
8	Luteolin hexoside pentoside III	nd^{a}	tr^{a}	tr^{a}	0.01 ± 0.00^{a}
12	Luteolin 7-O-(2-apiosyl)glucoside	nd^{a}	0.01 ± 0.00^{a}	0.01 ± 0.00^{a}	0.02 ± 0.00^{a}
15	Luteolin 7-O-(2-apiosyl-6-malonyl)glucoside	0.08 ± 0.01^{a}	0.12 ± 0.02^{ab}	0.16 ± 0.00^{b}	0.29 ± 0.02^{c}
	Total Luteolin derivates	**0.08 ± 0.01^{a}**	**0.15 ± 0.02^{b}**	**0.20 ± 0.00^{b}**	**0.37 ± 0.02^{c}**
	Total Flavonoids	**0.26 ± 0.02^{a}**	**0.38 ± 0.04^{b}**	**0.52 ± 0.02^{c}**	**0.90 ± 0.03^{d}**
1	Caffeic acid glucoside I	nd^{a}	tr^{a}	tr^{a}	tr^{a}
2	Caffeic acid glucoside II	tr^{a}	tr^{a}	0.01 ± 0.00^{a}	0.03 ± 0.00^{b}
	Total Caffeic acid derivates	**0.00 ± 0.00^{a}**	**0.00 ± 0.00^{a}**	**0.01 ± 0.00^{a}**	**0.03 ± 0.00^{a}**
3	CQA	0.01 ± 0.00^{a}	0.02 ± 0.00^{ab}	0.02 ± 0.00^{ab}	0.03 ± 0.00^{b}
	Total CQA derivates	**0.01 ± 0.00^{a}**	**0.02 ± 0.00^{ab}**	**0.02 ± 0.00^{ab}**	**0.03 ± 0.00^{b}**
	Total Phenolic acids	**0.01 ± 0.00^{a}**	**0.02 ± 0.00^{a}**	**0.03 ± 0.00^{a}**	**0.06 ± 0.00^{b}**

Different letters for each row indicate significant differences ($p \leq 0.05$) among samples.

nd: no detected, tr: traces.

Table 3. (Poly)phenolic compounds in raw and cooked cardoon (fried in olive oil, fried in sunflower oil and griddle). Results are expressed as mean ± standard deviation (mg/g sample dm).

Peak no.	Compound	Raw	Fried in olive oil	Fried in sunflower oil	Griddled
1	CQA I	0.03 ± 0.00^{a}	0.07 ± 0.00^{a}	0.08 ± 0.00^{a}	0.06 ± 0.00^{a}
2	CQA II	0.03 ± 0.01^{a}	0.03 ± 0.00^{a}	0.05 ± 0.00^{a}	0.08 ± 0.00^{a}
3	5- CQA	3.32 ± 0.15^{a}	2.84 ± 0.05^{a}	3.05 ± 0.05^{a}	7.29 ± 0.35^{b}
4	CQA III	0.08 ± 0.00^{c}	0.03 ± 0.00^{a}	0.04 ± 0.01^{b}	0.03 ± 0.00^{a}
	Total CQAs acids	**3.46 ± 0.02^{a}**	**2.98 ± 0.05^{a}**	**3.22 ± 0.05^{a}**	**7.46 ± 0.35^{b}**
6	1,3- diCQA	nd^{a}	nd^{a}	0.03 ± 0.00^{a}	0.02 ± 0.00^{a}
7	3,4- diCQA	tr^{a}	0.07 ± 0.00^{b}	0.10 ± 0.00^{c}	0.12 ± 0.00^{d}
8	1,4- diCQA	nd^{a}	0.07 ± 0.00^{b}	0.10 ± 0.01^{c}	0.12 ± 0.00^{d}
9	3,5- diCQA	0.90 ± 0.04^{a}	1.61 ± 0.01^{b}	2.01 ± 0.06^{c}	4.42 ± 0.15^{d}
10	1,5- diCQA	0.47 ± 0.02^{a}	1.08 ± 0.06^{b}	1.26 ± 0.02^{c}	2.10 ± 0.02^{d}
11	4,5- diCQA	0.03 ± 0.00^{a}	0.29 ± 0.02^{c}	0.25 ± 0.01^{b}	0.43 ± 0.01^{d}
	Total DiCQAs acids	**1.41 ± 0.06^{a}**	**3.12 ± 0.07^{b}**	**3.76 ± 0.08^{c}**	**7.21 ± 0.18^{d}**
12	SuccinyldiCQA I	0.13 ± 0.01^{a}	0.32 ± 0.01^{b}	0.40 ± 0.00^{c}	0.64 ± 0.00^{d}
13	SuccinyldiCQA II	0.18 ± 0.00^{b}	0.11 ± 0.00^{a}	0.13 ± 0.00^{a}	0.47 ± 0.01^{c}
14	SuccinyldiCQA III	0.01 ± 0.00^{a}	tr^{a}	tr^{a}	0.01± 0.00^{a}
16	DisuccinyldiCQA	0.02 ± 0.00^{a}	0.01 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02± 0.00^{a}
	Total SuccinyldiCQAs acids	**0.35 ± 0.02^{a}**	**0.45 ± 0.12^{b}**	**0.55 ± 0.00^{c}**	**1.14 ± 0.01^{d}**
	Total Chlorogenic acids	**5.22 ± 0.09^{a}**	**6.55 ± 0.13^{b}**	**7.53 ± 0.14^{b}**	**15.82 ± 0.54^{c}**
15	Apigenin glucoside	nd^{a}	nd^{a}	nd^{a}	tr^{a}
5	Luteolin glucoside	tr^{a}	tr^{a}	tr^{a}	0.02 ± 0.00^{b}
	Total Flavonoids	**tr^{a}**	**tr^{a}**	**tr^{a}**	**0.02 ± 0.00^{b}**

Different letters for each row indicate significant differences ($p \leq 0.05$) among samples.

Nd: no detected, tr: traces