

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

3 **RUNNING TITLE: Heat treatment on antioxidant capacity and phenolics of**
4 **vegetables**

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22 **ABSTRACT**

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

36 **KEYWORDS:** Phenolics; flavonoids; chlorogenic acids; antioxidants; vegetables; heat
37 treatment

38

39 **1. Introduction**

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

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

64 compounds can also be affected by thermal processes and, consequently the antioxidant
65 capacity of consumed vegetables too (Ramírez-Anaya, Samaniego-Sánchez, Castañada-
66 Saucedo, Villalón-Mir & de la Derrana, 2015). There are some studies that report the
67 effect of heat treatment on antioxidant activity and (poly)phenolic compounds in
68 vegetables. While boiling is the most investigated cooking method, few studies are
69 about frying process, both deep frying and pan frying (Palermo, Pellegrini & Fogliano,
70 2014) and as far as we know, only one study was found about the effect of griddling on
71 the antioxidant capacity of vegetables, (Jiménez-Monreal, García-Diz, Martínez-Tomé,
72 Mariscal & Murcia, 2009), but none on the (poly)phenols profile. However, results
73 reported on the effect of heat treatment on the (poly)phenolic compounds are not clear
74 cut. Onion is one of the most studied vegetables, nevertheless both losses and gains in
75 (poly)phenolic compounds after heat treatment are reported in the literature (Price,
76 Bacon & Rhodes, 1997; Crozier, Lean, Morag & Black, 1997; Ewald, Fjelkner-Modig,
77 Johansson, Sjöholm & Akesson, 1999; Lombard, Peffley, Geoffriau, Thompson &
78 Herring, 2005; Rhon, Buchner, Driemel, Rauser & Hroh, 2007; Rodrigues, Pérez-
79 Gregorio, García-Falcón & Simal-Gándara, 2009; Harris, Bruton, Tiwari & Cummins,
80 2015). The studies found in literature about effect of heat treatment on green pepper are
81 focused on antioxidant activity, but not on (poly)phenolic compound profiles changes
82 (Jiménez-Monreal et al. 2009) and up to our knowledge, this is the first time where the
83 influence of heat treatment on antioxidant capacity and (poly)phenol compounds of
84 cardoon stalks (*Cynara cardunculus* L.) has been studied. Therefore, the aim of this
85 work was to study the impact of three cooking heat treatments (frying in olive oil,
86 frying in sunflower oil and griddled) on the antioxidant capacity and (poly)phenolic
87 compound profiles of onion, green pepper and cardoon, commonly consumed as crude
88 in salads and cooked in several ways in the mediterranean diet.

89 2. Material and methods

90 2.1 Chemical and reagents

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

94 The methanol and ethanol were of analytical grade from Panreac (Barcelona, Spain).

95 The methanol (HPLC grade) was purchased from Panreac (Barcelona, Spain). Trolox
96 (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,2'-azinobis (3-
97 ethylbenzothiazonile-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-
98 picrylhydrazyl (DPPH·), as well as the standards used for identification and
99 quantification of phenolic compounds (quercetin, luteolin, isorhamnetin, 5-
100 caffeoylquinic acid and caffeic acid), were purchased from Sigma-Aldrich (Steinheim,
101 Germany).

102 2.2 Samples preparation

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

110 2.3. Vegetables extracts

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

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

119 2.4. Antioxidant capacity by ABTS assay

120 The ABTS antioxidant capacity was performed according to the method of Re,
121 Pellegrini, Proteggente, Pannala, Yang & Rice-Evans, (1999). The radicals ABTS^{•+}
122 were generated by the addition of 0.36 mM potassium persulfate to a 0.9 mM ABTS
123 solution prepared in phosphate buffered saline (PBS) (pH 7.4), and the ABTS^{•+} solution
124 was stored in darkness for 12 h. The ABTS^{•+} solution was adjusted with PBS to an
125 absorbance of 0.700 (± 0.020) at 734 nm in a 3 mL capacity cuvette (1 cm length) at 25
126 °C (Lambda 25 UV–VIS spectrophotometer, Perkin-Elmer Instruments, Madrid, Spain).
127 An aliquot of 100 μ L of each vegetable extract sample properly diluted in demineralized
128 water, was added to 2 mL of ABTS^{•+} solution. The absorbance was measured
129 spectrophotometrically at 734 nm after exactly 18 min. Calibration was performed with
130 Trolox solution (a water-soluble vitamin E analog), and the antioxidant capacity was
131 expressed as micromoles of Trolox equivalent per gram of dry matter sample (μ mol
132 Trolox/g dm).

133 2.5. Antioxidant capacity by DPPH assay

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

138 (± 0.020) at 515 nm in a 3 mL capacity cuvette (1 cm length) at 25 °C (Lambda 25 UV–
139 VIS spectrophotometer, Perkin-Elmer Instruments, Madrid, Spain). Vegetable extracts
140 were properly diluted in demineralized water prior to analysis. Samples (50 μ L) were
141 added to 1.95 mL of the DPPH[•] solution. After mixing, the absorbance was measured at
142 515 nm after exactly 18 min. Calibration was performed with Trolox solution (a water-
143 soluble vitamin E analog). The antioxidant capacity was expressed as micromoles of
144 Trolox equivalent per gram of dry matter sample (μ mol Trolox/g dm).

145 2.6. (Poly)phenolic compounds by UHPLC-PDA-HR-MS

146 (Poly)phenolic compounds were analysed using an UPLC with a PDA detector scanning
147 from 200-600 nm, equipped with an autosampler cooled at 4 °C (Dionex Ultimate 3000
148 RS, Thermo Corporation) and an ExactiveTM Orbitrap mass spectrometer fitted with a
149 heated electrospray ionization probe (HESI) (Thermo Fisher Scientific, San José, USA).
150 Chromatographic separation was performed at 40 °C on a Kinetex 5 μ m RP 250 x
151 4,6 mm reversed phase column (Phenomenex, Macclesfield, UK). Ten microliter of
152 each ethanolic extract was analysed using an 80 min 5 to 50 % gradient of acetonitrile in
153 0.1 % aqueous formic acid at a constant flow rate of 1 mL/min. After passing the PDA
154 flow cell, the eluate was split and 0.2 mL/min was directed to the mass spectrometer
155 with the HESI operating in negative ionization mode. Analysis was carried out in full-
156 scan (100-800 m/z) and full-scan with In-Source Collision-induced dissociation (CID)
157 (100-800 m/z; CID 25.0 eV). Capillary temperature was 300°C; sheath gas and
158 auxiliary gas were 60 and 20 units/min, respectively; source voltage was 4.0 kV.
159 Identification was achieved by comparing the exact mass and retention time with pure
160 reference standards. In absence of standards, compounds were tentatively identified by
161 comparing the theoretical exact mass of the molecular ion with the experimentally
162 measured accurate mass of the molecular ion. In addition identification was confirmed

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

173 2.7. Statistical analysis

174 Each parameter was analysed in triplicate. Results are shown as the mean ± standard
175 deviation (SD). One-way analysis of variance (ANOVA) was applied for each
176 parameter. A Tukey test was applied as *a posteriori* test with a level of significance of
177 95%. Principal Component Analysis (PCA), based on Pearson's correlation matrix, was
178 applied in order to study the effect of heat treatment. All statistical analyses were
179 performed using the STATA v.12.0 software package.

180

181 **3. Results and discussion**

182 Total of seven flavonoids were identified and quantified in onion samples. All of them
183 were glucosides of quercetin and isorhamnetin. Aglycones of these two flavonoids were
184 not detected. Other minor flavonoids such as kaempferol, luteolin and myricetin
185 derivatives, or phenolic acids identified in onion by other authors were not detected in our
186 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. Additionally, 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 decreased
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

212 during 5 minutes, whereas at higher frying times (15 minutes) decreased significantly
213 (Price et al. 1997). Lombard et al. (2005) also showed how different heat treatments
214 could affect phenolic content; baking or sauteing increased concentrations of
215 predominant quercetin derivatives in 25% and 7%, respectively, compared to raw onions
216 as in the present study, while boiling decreased total flavonoid concentration in 18%.
217 However, other authors reported that frying process induced 21-39% losses of
218 flavonoids in onion (Crozier et al. 1997; Ewald et al. 1999) while others reported no
219 significant changes in the total levels of quercetin diglucoside and monoglucoside
220 (Rodrigues et al. 2009). In the present study, all compounds tended to increase after
221 cooking process, but molar ratio of quercetin diglucoside/quercetin glucoside decreases
222 with the frying process. In onion fried in olive oil and in sunflower oil, quercetin
223 diglucoside/glucoside had ratios of 0.74 and 0.72, respectively, lower than that of the
224 raw onion (0.83). These results suggest that during frying process, glucosides of the
225 quercetin diglucosides are thermohydrolyzed producing the corresponding
226 monoglucoside as discussed by several authors (Rhon et al. 2007; Rodrigues et al.
227 2009). However in griddled onion, total onion quercetin diglucosides significantly
228 increased, probably due to the degradation of other quercetin derivatives such as quercetin
229 triglucoside, that may be strongly linked to other structures in the food matrix and thus
230 have not been fully extracted. The softening effect of the cooking process of vegetables,
231 in this case mainly griddling in onion, due to heat-induced wall and cells ruptures can
232 also affect (poly)phenolic extractability (Palermo et al., 2014; Harris et al. 2015).
233 Although some authors reported that quercetin monoglucoside can be also
234 deglycosylated by thermohydrolysis to the corresponding aglycon (Rohn et al., 2007,
235 Rodrigues et al. 2009), no quercetin or isorhamnetin aglycons were detected after
236 cooking process in the present work in agreement with Harris et al. (2015), probably

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

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

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

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

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

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

287 the phenolic compounds characteristics of onion and green pepper, the most abundant
288 (poly)phenols of cardoon were chlorogenic acids (four caffeoylquinic acids (CQA), six
289 dicaffeoylquinic acids (diCQA), three succinyldicaffeoylquinic acids (succinyldiCQA)
290 and one disuccinyldicaffeoylquinic acid (disuccinyldiCQA) (Table 3). These last
291 compounds were reported for the first time in raw cardoon leaves (*C. cardunculus L.*)
292 by Pinelli et al. (2007). In the cardoon stalks of the present study, 5-CQA, 3,5-diCQA
293 and 1,5-diCQA were the most abundant compounds, accounting for 80-90% of total
294 (poly)phenolic compounds detected by HPLC in all samples. Traces of some flavonoids
295 were also detected in cardoon, mainly in griddled one. Up to our knowledge, this is the
296 first time where the influence of heat treatment on antioxidant capacity and
297 (poly)phenol compounds of cardoon stalks (*Cynara cardunculus L.*) has been studied.
298 The total amount of (poly)phenolic compounds identified by HPLC increased after heat
299 treatment. Chlorogenic acids can be found free, but also ionically or covalently attached
300 to other food structures, like melanoidin. DiCQAs, where the addition of a caffeic acid
301 moiety increases the hydroxyl groups, can ionically interact with other macromolecules
302 (Monente, Ludwig, Irigoyen, de Peña & Cid, 2015). The softening effect of the cooking
303 process of vegetable due to heat-induced wall and cells ruptures can affect
304 (poly)phenolic extractability (Palermo et al., 2014), so heat treatment could hydrolyzed
305 and release those compounds from the food matrix. The increment in total
306 (poly)phenolic compounds was higher in griddled cardoon (203.06%) than in cardoon
307 fried in sunflower oil (44.25%) and in cardoon fried in olive oil (25.47%). This could be
308 due to the higher temperature applied during griddled treatment than that applied during
309 frying processes, which might favor the release of these compounds from the food
310 matrix, specially 5CQA which increased dramatically after griddling process probably
311 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 scavenging 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 isorhamnetin derivatives 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 derivatives 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 comparison 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 bioaccessibility, 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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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 \pm standard deviation (mg/g sample dm).

Peak no.	Compound	Raw	Fried in olive oil	Fried in sunflower oil	Griddled
1	Quercetin triglucoside	0.01 \pm 0.00 ^a	0.01 \pm 0.00 ^a	0.01 \pm 0.00 ^a	0.01 \pm 0.00 ^a
2	Quercetin diglucoside I	0.22 \pm 0.00 ^a	0.30 \pm 0.01 ^a	0.24 \pm 0.03 ^a	0.35 \pm 0.06 ^a
3	Quercetin diglucoside II	0.35 \pm 0.00 ^a	0.42 \pm 0.01 ^b	0.38 \pm 0.02 ^{ab}	0.56 \pm 0.03 ^c
5	Quercetin glucoside I	0.04 \pm 0.00 ^b	0.02 \pm 0.00 ^a	0.02 \pm 0.00 ^a	0.03 \pm 0.00 ^{ab}
6	Quercetin glucoside II	0.64 \pm 0.01 ^a	0.95 \pm 0.01 ^c	0.83 \pm 0.00 ^b	1.02 \pm 0.04 ^c
	Total Quercetin derivates	1.26 \pm 0.01^a	1.71 \pm 0.03^{bc}	1.48 \pm 0.05^{ab}	1.96 \pm 0.12^c
4	Isorhamnetin diglucoside	0.02 \pm 0.00 ^a	0.12 \pm 0.02 ^{bc}	0.09 \pm 0.02 ^b	0.17 \pm 0.02 ^c
7	Isorhamnetin glucoside	0.08 \pm 0.00 ^a	0.01 \pm 0.00 ^a	0.01 \pm 0.00 ^a	0.01 \pm 0.00 ^a
	Total Isorhamnetin derivates	0.10 \pm 0.01^a	0.13 \pm 0.02^{ab}	0.10 \pm 0.02^a	0.18 \pm 0.01^b
	Total Flavonoids	1.36 \pm 0.01^a	1.83 \pm 0.05^{bc}	1.57 \pm 0.07^{ab}	2.14 \pm 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 \pm 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 \pm 0.00 ^a	0.01 \pm 0.00 ^a	0.01 \pm 0.00 ^a
11	Quercetin rhamnoside glucoside II	nd ^a	0.01 \pm 0.00 ^a	0.01 \pm 0.00 ^a	0.02 \pm 0.00 ^b
6	Quercetin 3-sambubioside-7-rhamnoside	0.01 \pm 0.00 ^a	0.01 \pm 0.00 ^a	0.01 \pm 0.00 ^a	0.02 \pm 0.00 ^a
14	Quercetin rhamnoside	0.17 \pm 0.00 ^a	0.19 \pm 0.02 ^{ab}	0.24 \pm 0.01 ^b	0.42 \pm 0.01 ^c
13	Quercetin glucoside	0.01 \pm 0.00 ^a	0.02 \pm 0.00 ^a	0.04 \pm 0.00 ^{ab}	0.06 \pm 0.00 ^b
	Total Quercetin derivates	0.18 \pm 0.41^a	0.23 \pm 0.02^a	0.32 \pm 0.02^b	0.53 \pm 0.01^c
9	Luteolin glucoside I	nd ^a	tr ^a	0.01 \pm 0.00 ^a	0.01 \pm 0.00 ^a
10	Luteolin glucoside II	nd ^a	tr ^a	0.01 \pm 0.00 ^a	0.02 \pm 0.00 ^a
5	Luteolin hexoside pentoside I	nd ^a	tr ^a	0.01 \pm 0.00 ^a	0.01 \pm 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 \pm 0.00 ^a
12	Luteolin 7-O-(2-apiosyl)glucoside	nd ^a	0.01 \pm 0.00 ^a	0.01 \pm 0.00 ^a	0.02 \pm 0.00 ^a
15	Luteolin 7-O-(2-apiosyl-6-malonyl)glucoside	0.08 \pm 0.01 ^a	0.12 \pm 0.02 ^{ab}	0.16 \pm 0.00 ^b	0.29 \pm 0.02 ^c
	Total Luteolin derivates	0.08 \pm 0.01^a	0.15 \pm 0.02^b	0.20 \pm 0.00^b	0.37 \pm 0.02^c
	Total Flavonoids	0.26 \pm 0.02^a	0.38 \pm 0.04^b	0.52 \pm 0.02^c	0.90 \pm 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 \pm 0.00 ^a	0.03 \pm 0.00 ^b
	Total Caffeic acid derivates	0.00 \pm 0.00^a	0.00 \pm 0.00^a	0.01 \pm 0.00^a	0.03 \pm 0.00^a
3	CQA	0.01 \pm 0.00 ^a	0.02 \pm 0.00 ^{ab}	0.02 \pm 0.00 ^{ab}	0.03 \pm 0.00 ^b
	Total CQA derivates	0.01 \pm 0.00^a	0.02 \pm 0.00^{ab}	0.02 \pm 0.00^{ab}	0.03 \pm 0.00^b
	Total Phenolic acids	0.01 \pm 0.00^a	0.02 \pm 0.00^a	0.03 \pm 0.00^a	0.06 \pm 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 \pm standard deviation (mg/g sample dm).

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

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

Nd: no detected, tr: traces



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