*

1 **The effects of heat treatment on the phenolic composition and antioxidant capacity of red wine pomace**
2 **seasonings.**
3
4 Running title:
5 **Impact of heating on the polyphenols of red wine pomace seasonings.**
6
7 Raquel Del Pino-García [a], María L. González-SanJosé [a], María D. Rivero-Pérez [a], Javier García-Lomillo [a]
8 and Pilar Muñiz [a,]*
9
10 [a] Department of Food Biotechnology and Science, Faculty of Sciences, University of Burgos, Plaza Misael
11 Bañuelos, 09001, Burgos, Spain.
12
13 ***Corresponding author**: Dr. Pilar Muñiz Rodríguez, Plaza Misael Bañuelos, Facultad de Ciencias,
14 Departamento de Biotecnología y Ciencia de los Alimentos, 09001, Burgos, Spain.
15 ***E-mail***: pmuniz@ubu.es
16 ***Phone***: +34-947258800 Ext. 8210
17 ***Fax***: +34-947258831
18
19 **Email addresses:** Raquel Del Pino-García (rdpino@ubu.es), María L. González-SanJosé (marglez@ubu.es),
20 María D. Rivero-Pérez (drivero@ubu.es), Javier García-Lomillo (jglomillo@ubu.es), Pilar Muñiz
21 (pmuniz@ubu.es).

This document is the Submitted Manuscript version of a Published Work that appeared in final form in Food Chemistry. To access the final edited and published work see http://dx.doi.org/10.1016/j.foodchem.2016.10.113.”

© 2016. This manuscript version is made available under the CC-BY-NC-ND 4.0 license http://creativecommons.org/licenses/by-nc-nd/4.0/

Abstract

The impact of thermal processing on the phenolic profile and total antioxidant capacity (TAC) of powdered red wine pomace seasonings (RWPSs) obtained from different sources (seedless:Sk-S; whole:W-S; seeds:Sd-S) was assessed. High contents in anthocyanins, flavonol-3-*O*-glycosides, phenolic acids and flavan-3-ols were found in Sk-S, whereas flavan-3-ols and phenolic acids were the main compounds identified in Sd-S (HPLC-DAD analysis). Reductions in the anthocyanidin and flavonol-3-ol contents mainly determined the effects of heating on the total phenolic contents (Sk-S:-29.4%; W-S:-28.0%; Sd-S:-5.78%), although heating affected positively the phenolic acid and flavonol aglycon contents. Slight TAC decreases were observed in the RWPS-derived extracts (classical Folin-Ciocalteu and ABTS assays), detecting higher TAC reductions when the powdered RWPSs were used directly as samples (QUENCHER approach). In conclusion, there is little evidence against submitting RWPSs to thermal processing, as heating affects differently each type of phenolic compound and does not induce very severe TAC decreases in these seasonings.

Keywords

phenolic profile; QUENCHER; thermal processing; total antioxidant capacity; total polyphenol content; winery by-products.

39 **1. Introduction**

40

41 Wine pomace comprises the skins, seeds and stems left after pressing grapes in the winemaking process.
42 This waste is characterized by a high polyphenol content because of the only partial extraction of these
43 compounds into wine during winemaking (Delgado Adámez, Gamero Samino, Valdés Sánchez, & González-
44 Gómez, 2012; Sacchi, Bisson, & Adams, 2005), although the different groups of phenolic compounds are not
45 uniformly distributed among the components of wine pomace (Kammerer, Claus, Carle, & Schieber, 2004;
46 Pinelo, Arnous, & Meyer, 2006; Rockenbach et al., 2011). The phytochemicals present in wine pomace are
47 known to have antioxidant, preservative and health-promoting effects in different biological and food
48 systems (Andersen & Markham, 2005; Fontana, Antoniolli, & Bottini, 2013; Teixeira et al., 2014). As such,
49 the recycling of winery by-products represents an opportunity to provide valuable raw materials for the
50 pharmaceutical, cosmetics, nutraceuticals, and food industries, thereby contributing to reducing the costs and
51 environmental impact linked to the disposal of these residues in wine producing regions (Arvanitoyannis,
52 Ladas, & Mavromatis, 2006; Teixeira et al., 2014).
53 Most of the polyphenol-rich products derived from winemaking residues are obtained by applying extraction
54 techniques (Louli, Ragoussis, & Magoulas, 2004; Monagas, Garrido, Bartolomé, & Gómez-Cordovés, 2006).
55 An alternative to such extractions is to obtain powdered products derived directly from winery by-products
56 (García-Lomillo, González-Sanjosé, Del Pino-García, Rivero-Pérez, & Muñiz, 2014; Saura-calixto, 1998).
57 This strategy is being studied by our research group to develop wine pomace products with potential uses as
58 seasonings and dietary supplements in the food and nutraceuticals industry (González-Sanjosé, García-
59 Lomillo, Del Pino-García, Muñiz-Rodríguez, & Rivero-Pérez, 2013). However, the main shortcoming of the
60 direct use of this winery waste is the presence of undesirable microorganisms (residual yeast and bacteria
61 from the vinification process). A drying pre-treatment is usually applied to wet wine pomace to extend its
62 storage stability until final transformation into the desired extracts (Pedroza, Carmona, Pardo, Salinas, &
63 Zalacain, 2012; Spigno, Tramelli, & De Faveri, 2007) or powdered products (Larrauri, Rupérez, & Saura-
64 Calixto, 1997). However, the long-term stability of these products is questionable and pre-treatment may not
65 be sufficient to reduce the microbial loads to acceptable values to guarantee subsequent food safety when the
66 seasonings are incorporated into fresh foodstuffs. As such, an stabilization step was included in the
67 manufacturing process of these seasonings (González-Sanjosé et al., 2013), selecting heat treatment at 90 ºC

68 for 90 min as the most suitable method for achieving complete microbial inactivation (García-Lomillo et al.,
69 2014).
70 The sensitivity of wine pomace polyphenols to high temperatures and the effect of this on total antioxidant
71 capacity (TAC) have been subject of numerous research studies, most of which concerned wine pomace
72 extracts (Chamorro, Goñi, Viveros, Hervert-Hernández, & Brenes, 2012; Davidov-Pardo, Arozarena, &
73 Marín-Arroyo, 2011; Larrauri, Sanchez-Moreno, & Saura-Calixto, 1998; Monrad, Howard, King, Srinivas,
74 & Mauromoustakos, 2010; Palma, Piñeiro, & Barroso, 2001; Pinelo, Tubilar, Jerez, Sineiro, & Nuñez, 2005;
75 Sólyom, Solá, Cocero, & Mato, 2014; Volf, Ignat, Neamtu, & Popa, 2014). However, only a few studies
76 have investigated the detrimental or positive impact of submitting raw wine pomace materials to heat
77 treatment (Chamorro et al., 2012; Khanal, Howard, & Prior, 2010; Larrauri et al., 1997; Pedroza et al., 2012;
78 Ross, Hoye, & Fernandez-Plotka, 2011; Sólyom et al., 2014), and none of these works has assessed products
79 obtained from the skins, seeds and whole wine pomace, to determine the influence of these different matrices
80 on the impact of high temperatures. In addition, the effects of heating wine pomace and derived products
81 have been evaluated by classical (C-) TAC methodologies for extracts, but not using QUENCHER (Q-)
82 approaches for wine pomace powders. In this regard, Q-TAC assays have been suggested as suitable
83 methods for the assessment of foodstuffs in which insoluble materials may play an important antioxidant role
84 (Del Pino-García, García-Lomillo, Rivero-Pérez, González-Sanjosé, & Muñiz, 2015; Gökmen, Serpen, &
85 Fogliano, 2009).
86 In view of the above, this study aimed to characterize and assess the impact of heat treatment on the phenolic
87 profile (individual compounds and main classes) and antioxidant capacity (using C-TAC and Q-TAC
88 approaches) of different wine pomace seasonings.
89
90 **2. Materials and methods**
91
92 *2.1. Chemicals*
93 Pure phenolic compound standards (caffeic acid, caftaric acid, catechin, coutaric acid, ellagic acid,
94 epicatechin, ethyl gallate, fertaric acid, ferulic acid, gallic acid, kaempferol, kaempferol-3-*O*-glucoside,
95 kaempferol-3-*O*-rutinoside, myricetin, myricetin-3-*O*- rhamnoside, *p*-coumaric acid, *p*-OH-benzoic acid,
96 procyanidin B1, procyanidin B2, protocatechuic acid, quercetin, quercetin-3-*O*-rutinoside, salicylic acid,
97 syringic acid, *t*-resveratrol, *t*-piceid and vallinic acid) were purchased from Sigma-Aldrich Chemical Co. (St.

98 Louis, MO, USA). Anthocyanin standards (cyanidin chloride, delphinidin chloride, malvidin chloride,
99 pelargonidin chloride, peonidin chloride and petunidin chloride) and pelargonidin-3-*O*-glucoside were
100 obtained from Extrasynthese (Genay, France). Unless otherwise stated, all other chemicals and reagents were
101 obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA), Panreac (Barcelona, Spain) or Fisher
102 Scientific (Loughborough, UK).

103

104 *2.2. Samples*

105

106 *2.2.1. Red wine pomace seasonings (RWPSs)*

107 Red wine pomace from the vinification of *Vitis vinífera* L. *cv. Tempranillo* was kindly supplied by several
108 wineries situated in Burgos (Spain). All the wine pomace was mixed and dehydrated in an oven at a
109 temperature of less than 60 ºC to a final moisture content of less than 10%. Dried materials were then
110 separated, milled and sieved to obtain three RWPSs: one from seedless wine pomace (enriched in skins),
111 named Sk-S; another from whole wine pomace, named W-S; and a third derived from the isolated seeds,
112 named Sd-S. The particle size of the powdered products was less than 0.250 (Sk-S and W-S) and 0.355 mm
113 (Sd-S). Some of the products were then heated at 90 ºC for 90 min (García-Lomillo et al., 2014). The non-
114 treated products were identified as NT and the heat-treated products as HT.

115

116 *2.2.2. RWPS extracts*

117 Extracts from the different RWPSs were obtained by liquid extraction using MeOH:HCl 37% (97:3, v/v) as
118 solvent. A 1 ± 0.005 g sample of each product was weighed in triplicate, hydrated and mixed with 15 mL of
119 the solvent. After extraction at 25 ºC for 24 hour with continuous stirring (90 rpm), the mixture was filtered
120 and the residue washed twice with 5 mL of extraction solvent. The RWPS extracts were finally made up to
121 25 mL with the same solvent and stored at 4 ºC until used.

122 Different extracts were obtained for the anthocyanidin analysis using the method described in Rodríguez-
123 Mateos et al., (2014) for the extraction of these compounds. Thus, 0.5 ± 0.005 g of each product was
124 extracted three times with 5 mL of acidified methanol (0.1% HCl in MeOH) and pelargonidin-3-*O*-glucoside
125 (300 µL, 1 mM) added to the samples as recovery standard. They were then vortexed for 5 min, sonicated for
126 5 min in an ultrasonic bath, and centrifuged for 15 min at 1800g. The supernatants were combined, diluted
127 1:1 with 5 M HCl and heated at 90 °C for 1 h for anthocyanin hydrolysis.

128

129 *2.3. HPLC phenolic compounds analysis*

130

131 *2.3.1. Phenolic acids, stilbenes, flavan-3-ols and flavonols*

132 The RWPS extracts were analyzed according to a slightly modified version of the method described by
133 Pérez-Magariño et al., (2008). Identification and quantification of phenolic compounds was carried out using
134 analytical reversed-phase HPLC on an Agilent 1100 series HPLC system (Agilent Technologies Inc., Palo
135 Alto, CA, USA) coupled to a diode array detector. A Spherisorb3® ODS2 reversed phase C18 column (250
136 mm x 4.6 mm, 3 μm particle size; Waters Cromatografia S.A., Barcelona, Spain) was used. The
137 chromatographic conditions were as follows: flow, 0.6 mL/min; injection volume, 100 μL; mobile phases: A,
138 water:glacial acetic acid (98:2, v/v); B, water:acetonitrile:glacial acetic acid (78:20:2, v/v/v); C, acetonitrile.
139 The solvent gradient used was: 0-25 min, linear gradient from 0-100% to 25-75% of B in A; 25-60 min,
140 linear gradient from 25-75% to 70-30% of B in A; 60-100 min, linear gradient from 70-30% to 100-0% of B
141 in A; 100-120 min, 100% B; 120-130 min; linear gradient from 0-100% to 100-0% of C in B; 130-140 min,
142 100% C; 140-150 min; linear gradient from 100-0% to 0-100% of C in A. The eluent was monitored at 254,
143 280, 320, 360, and 520 nm, with compound spectra being obtained between 220 and 600 nm. Samples were
144 injected in duplicate, and calibration was performed by injecting the standards three times at five different
145 concentrations. Peak identification was performed by comparison of retention times and diode array spectral
146 characteristics with the standards. The results were expressed in μg/g seasoning.

147

148 *2.3.2. Anthocyanidins*

149 The RWPS extracts obtained for anthocyanidin analysis were identified and quantified using an Agilent 1100
150 series HPLC system (Agilent Technologies) equipped with a diode array detector. The column was a Nova-
151 Pak reverse phase C18 (250 mm x 4.6 mm (id), 4 μm particle size; 30 ºC; Waters LTd., Elstree, U.K.). The
152 chromatographic conditions were as follows: flow, 0.8 mL/min; injection volume, 50 μL; Elution solvents:
153 A, water:formic acid (99.9:0.1, v/v); B, acetonitrile. The solvent gradient used was: 0-20 min, linear gradient
154 from 10-90% to 30-70% of B in A; 20-25 min, linear gradient from 30-70% to 80-20% of B in A; 25-30 min,
155 80-20% of B in A; 30-31min, linear gradient from 80-20% to 10-90% of B in A; 31-40 min, 10-90% of B in
156 A. The eluent was monitored at 520 nm, with compound spectra being obtained between 220 and 600 nm.
157 Samples were injected in duplicate, and calibration curves were obtained by injecting the anthocyanidin

158 standards three times at five different concentrations. Peak identification was performed by comparison of
159 retention times and diode array spectral characteristics with the standards. The results were expressed as µg/g
160 seasoning.
161
162 *2.4. Total antioxidant capacity (TAC)*
163 The classical (C-) and QUENCHER (Q-) versions of TAC assays were adapted to a 10 mL final reaction
164 volume from the FC (Folin-Ciocalteu) method developed by Singleton and Rossi, (1965) and the ABTS
165 (2,2'-azinobis 3-ethylbenzothiazoline-6-sulfonic acid) method described by Re et al., (1999).
166
167 *2.4.1. FC assays*
168 *C-FC*: A volume of 0.2 mL of the RWPS extracts was mixed with 0.2 mL of FC reagent and allowed to react
169 for 5 min. Then, 4 mL of a 0.7 M sodium carbonate solution was added and the final volume was made up to
170 10 mL with Milli-Q (MQ) water. The tubes were incubated for 1 hour in an orbital shaker and the
171 absorbance at 750 nm measured using a UV-vis spectrophotometer U-2000 (Hitachi, Ltd., Hubbardston,
172 MA, USA). The FC index was expressed as µmol of gallic acid equivalents (GAE)/g of product using a
173 linear calibration curve obtained with different quantities of the standard.
174 *Q-FC*: The protocol described in Del Pino-García et al., (2015), which is the same as the C-FC method but
175 substituting the volume of RWPSs extracts by 1 ± 0.005 mg of RWPSs and 0.2 mL of MQ water, was
176 applied.
177
178 *2.4.2. ABTS assays*
179 *C-ABTS*: A stock $ABTS^{\cdot+}$ solution was prepared by treating a 7 mM aqueous ABTS solution with 2.45 mM
180 $K_2O_8S_2$ in a 1:1 (v/v) ratio. Before the assay, the stock $ABTS^{\cdot+}$ solution was diluted with MQ water to obtain
181 a working solution with an absorbance value of 0.70 ± 0.02 at 734 nm. A volume of 0.2 mL of the RWPS
182 extracts was then mixed with the $ABTS^{\cdot+}$ working solution to reach a final volume of 10 mL. The
183 absorbance at 734 nm was measured after incubation fro 30 min in the dark with continuous stirring. The
184 results were expressed as µmol of Trolox equivalents (TE)/g of product by means of a dose-response curve
185 for different amounts of Trolox.
186 *Q-ABTS*: The method described in Del Pino-García et al., (2015), which is the same protocol as the C-ABTS
187 assay but replacing the 0.2 mL of RWPS extracts with 1 ± 0.005 mg of RWPSs, was applied.

188

189 *2.5. Statistical analysis*

190 Statistical analysis was performed using Statgraphics® Centurion XVI, version 16.2.04 (Statpoint

191 Technologies Inc., Warranton, VA, USA). Data were subjected to a one-way analysis of variance (ANOVA)

192 using Fisher's least significant difference (LSD) to detect significant differences between the products (Sk-S,

193 W-S, and Sd-S). Student's t-test was used to determine significant differences between NT and HT for each

194 type of seasoning. Linear correlations were established by determining Pearson's correlation coefficients. All

195 analyses were performed in triplicate. Three levels of significance were considered for all statistics: $p < 0.05$,

196 $p < 0.01$, and $p < 0.001$.

197

198 **3. Results**

199

200 *3.1. Identification and quantification of phenolic compounds*

201

202 *3.1.1. Differences in the phenolic profile between RWPSs (Sk-S, W-S, and Sd-S)*

203 **Table 1** shows the concentration of several phenolic compounds present in seasonings obtained from

204 different wine pomace materials (seedless, whole, and seeds) before and after being submitted to heat

205 treatment. As can be seen, the trends in the compositional variation between Sk-S, W-S, and Sd-S found in

206 NT were generally similar to those observed in HT.

207 The total phenolic compounds identified in the HPLC analysis for the RWPS are given at the end of **Table 1**.

208 The amount of such compounds in Sk-S was almost 1.2-fold higher than in W-S, and nearly 2.4- and 1.8-fold

209 higher than in Sd-S for NT and HT, respectively.

210 With regard to the total compounds grouped in phenolic classes, it can be seen that anthocyanidins were the

211 most abundant compounds detected in Sk-S, representing around 79.6% (NT) and 72.0% (HT) of the total.

212 The concentration of these compounds in Sk-S was around 1.4-fold higher than in W-S, with more marked

213 differences being found when compared to Sd-S (nearly 21.7- and 19.3-fold higher in NT and HT,

214 respectively). Furthermore, Sk-S was also richer than W-S and Sd-S in flavonols, phenolic acids, and

215 stilbenes. In contrast, the flavan-3-ols identified in Sd-S were the main contributors in these products,

216 representing approximately 72.5% (NT) and 70.4% (HT) of all phenolic compounds. For W-S,

217 anthocyanidins represented around 68.6% (NT) and 57.4% (HT), whereas flavan-3-ols represented about
218 17.9% (NT) and 21.4% (HT) of the total.
219 Considering the subgroups and individual phenolic compounds within the phenolic classes, hydroxybenzoic
220 acids contributed more to the total phenolic acid content than hydroxycinnamic acids in all RWPSs. The total
221 hydroxybenzoic and hydroxycinnamic acids contents both followed the same trend between RWPSs as that
222 observed for total phenolic acids (Sk-S > W-S = Sd-S for NT; Sk-S > W-S > Sd-S for HT), with the
223 exception of the content in hydroxycinnamic acids for HT, where no significant differences between the
224 seasonings were found. Gallic acid, and ethyl gallate were more abundant in Sd-S (Sk-S < W-S < Sd-S),
225 whereas ellagic acid was not detected. With regard to hydroxycinnamic acids, it was also notable that ferulic
226 acid was not found in NT for Sd-S, whereas small amounts were detected after heat treatment. The highest
227 quantities of the rest of non-esterified forms were generally found in Sd-S whereas Sk-S showed the highest
228 contents in the tartaric acid-derivatives.
229 As regards the flavan-3-ol subgroups, a smaller quantity of monomers (catechin and epicatechin) than dimers
230 (procyanidins B1 and B2) was found for Sk-S, whereas the opposite was observed for Sd-S.
231 The total contents in flavonol-3-*O*-glycosides were higher than in flavonol aglycons for Sk-S and W-S. The
232 same trend observed for the total flavonol content (Sk-S > W-S > Sd-S) was generally found for both
233 aglycons and 3-*O*-glycosides, with the exceptions of kaempferol and quercetin.
234 Malvidin was the most abundant anthocyanidin for all RWPSs, although important quantities of delphinidin
235 and petunidin were also found for Sk-S and W-S.
236
237 *3.1.2. Changes in the phenolic profile induced by heat treatment*
238 The differences in the individual phenolic composition of HT with respect to NT for each type of wine
239 pomace seasoning are presented in **Table 2** as percentage (%) concentration changes.
240 Heat treatment resulted in a significant decrease in the total amount of phenolic compounds detected in the
241 analysis, with this decrease being specially marked in Sk-S and W-S (Sk-S = W-S < Sd-S, $p < 0.01$).
242 A significant and positive effect of the heat treatment was observed for the total phenolic acid and flavonol
243 contents. In contrast, heating negatively affected the total stilbene, flavan-3-ol, and anthocyanidin contents.
244 The losses of stilbenes and flavan-3-ols were similar for the three types of RWPSs. Anthocyanidins were the
245 most severely affected compounds by the high temperatures, showing that the negative effect was around
246 10.7% less marked for Sd-S than for W-S.

247 The heat treatment had a higher positive effect on the total hydroxybenzoic acid content for Sk-S and W-S
248 than for Sd-S (Sk-S = W-S > Sd-S, $p < 0.05$), whereas the total hydroxycinnamic acid content remained
249 stable despite heat treatment. p-OH-benzoic acid and ethyl gallate contents decreased significantly in Sk-S
250 and W-S. In contrast, a positive effect of exposure to high temperatures was found for the remaining
251 hydroxybenzoic acids, with the % increase in salicylic and protocatechuic acids contents for Sk-S, and gallic
252 acid content for Sd-S, exhibiting the most marked changes. While heat treatment negatively affected the
253 amount of *p*-coumaric in Sd-S, this hydroxycinnamic acid was greatly increased in Sk-S and W-S. The
254 increase in ferulic acid content was also notable in Sk-S and W-S. In contrast, significant decreases in the
255 concentration of coutaric acid (Sk-S and W-S) and fertaric acid (Sk-S) were found.
256 The effects of heating on the total flavan-3-ol monomer and dimer contents were similar to the general
257 detrimental effect found for flavan-3-ols as a whole (Sk-S = W-S = Sd-S). The most significant % decrease
258 was observed in both catechin and epicatechin for W-S. A significant decrease of procyanidin B2 was
259 observed for Sk-S and W-S, with this decrease being more important for Sk-S than for W-S and Sd-S.
260 In the case of flavonols, the aglycon content was much more positively influenced by heat treatment than the
261 3-*O*-glycoside content. The increase in total flavonol aglycon content was higher for Sd-S than for Sk-S and
262 W-S ($p < 0.05$), with the increase in kaempferol and myricetin contents being also the most marked for Sd-S,
263 while quercetin only increased significantly in Sk-S. Heat treatment had no effect in the total flavonol-3-*O*-
264 glycoside content for Sk-S, whereas the content of these compounds rose significantly for W-S and Sd-S
265 (Sk-S < W-S = Sd-S, $p < 0.01$). The decreases in kaemferol-3-*O*-rutinoside and kaempferol-3-*O*-glucoside
266 contents for Sk-S, and the increase in myricetin-3-*O*-rahmnoside content for Sd-S, were the only significant
267 % changes observed.
268 The most notable decreases in anthocyanidin contents upon heat treatment were found for delphinidin (W-S
269 and Sd-S), peonidin (Sk-S) and malvidin (Sk-S and W-S). There was no significant change in cyanidin
270 content due to the exposure to high temperatures, although the comparison of the products showed a more
271 detrimental effect for Sk-S and W-S than for Sd-S ($p < 0.05$). An opposite effect was found for the
272 delphinidin content, whereas no differences between the RWPSs were obtained for peonidin and malvidin.
273 The marked reduction in malvidin content (around -37.0 %) due to heat treatment was mainly responsible for
274 the decrease in total anthocyanidin content.
275
276 *3.2. Changes in total antioxidant capacity (TAC)*

277 The FC and ABTS assays were used to assess the TAC for RWPS extracts, using C-TAC protocols, as well
278 as directly for the powdered products, applying the Q-TAC methods. As noted previously (Del Pino-García
279 et al., 2015), the results obtained with each method are influenced by the solubility of the standards in the
280 solvent or reaction medium used.
281 The C-FC and C-ABTS values (**Figure 1**A and C, respectively) showed similar trends for all RWPSs (both
282 NT and HT), with Sd-S exhibiting the highest C-TAC and Sk-S the lowest. In contrast, this trend was not
283 observed when using the Q-TAC methods. Similar results were obtained for Sk-S and W-S in the Q-FC
284 assay (**Figure 1**B), with both these samples giving a higher Q-FC index than Sd-S (both NT and HT). The
285 same trend was found for HT seasonings when using the Q-ABTS method, while W-S gave the highest value
286 of the three NT seasonings (**Figure 1**D).
287 With regard to the % change in TAC between HT and NT, greater differences were found between the assays
288 (**Table 3**). The antioxidant capacity of Sd-S was not significantly affected by heating according to the results
289 of the C-FC assay, whereas effects of heat treatment were observed for the remaining RWPSs and when
290 using other assays. The TAC reductions were generally more significant when using the Q-TAC than the C-
291 TAC methods, with the most significant % change being observed for Sk-S and W-S (Q-FC assay) and W-S
292 (Q-ABTS assay). A different trend between the RWPSs as regards the impact of heating was shown for each
293 method.
294
295 *3.3. Correlations*
296 Two different correlation studies were performed with data obtained in this study. Firstly, Pearson's
297 correlation coefficients were determined for the TAC values and the main phenolic class contents (**Table
298 4**A). Strong and positive correlations were found between the C-FC and C-ABTS methods, and also between
299 the Q-FC and Q-ABTS assays. However, both C-TAC assays were negatively related to the Q-FC method,
300 and unrelated to the Q-ABTS assay. The C-FC and C-ABTS methods were both highly and positively
301 correlated with the total flavan-3-ol content, whereas a negative correspondence was detected with the
302 remaining phenolic class and total phenolic compound contents. In contrast, the Q-FC method exhibited the
303 opposite trend, although it was not related to the total phenolic acid content.
304 Secondly, correlations were calculated using the % change for HT with respect to NT (**Table 4**B). For the
305 TAC assays, only the C-FC and C-ABTS methods were mutually positively correlated, whereas the C-ABTS
306 assay was negatively correlated to the Q-ABTS method. The C-FC assay was strongly and positively

307 correlated to the total flavan-3-ol and total phenolic compound contents, with a positive correlation to the
308 total flavonol and anthocyanidin contents also being found. The positive correlation between the C-ABTS
309 method and total stilbene and flavan-3-ol contents was also significant. In contrast, no positive correlations
310 between the Q-TAC assays and the phenolic classes were observed.
311
312 **4. Discussion**
313
314 The interest in a more efficient exploitation of winemaking by-products has led to innovative alternatives,
315 such as the use of wine pomace-derived products as food ingredients (González-Sanjosé et al., 2013). The
316 heat treatment required during the microbial stabilization step in the RWPS manufacturing process made it
317 necessary to characterize the phenolic profile and antioxidant capacity of the non-treated (NT) and heat-
318 treated seasonings (HT) obtained from the different wine pomace constituents (Sk-S, W-S, and Sd-S).
319
320 *4.1. Differences between Sk-S, W-S, and Sd-S.*
321 The individual phenolic content of wine pomace depends on numerous viticulture and wine-making factors,
322 such as the grape variety (Sri Harsha, Gardana, Simonetti, Spigno, & Lavelli, 2013), cultivation conditions
323 (Kammerer et al., 2004), and technological parameters applied during vinification (Fontana et al., 2013;
324 Sacchi et al., 2005). Furthermore, many of the polyphenols present in wine pomace are entrapped or bound
325 to the cell wall matrices, especially those contained in the seed portion, and thereby are not easily extracted
326 unless aggressive treatments are applied (Arranz, Silván, & Saura-Calixto, 2010).
327 In general, the distribution of phenolic classes and individual compounds in the RWPSs were in line with
328 those published previously(Alonso et al., 2002; Chamorro et al., 2012; Delgado Adámez et al., 2012;
329 Monagas, Gómez-Cordovés, Bartolomé, Laureano, & Da Silva, 2003; Rockenbach et al., 2011), as the skin
330 portion of wine pomace is known to be a rich source of anthocyanins, phenolic acids, flavan-3-ols, and
331 flavonol-3-*O*-glycosides, whereas gallic acid and flavan-3-ol monomers and oligomers are mainly present in
332 the seed portion. The phenolic contents observed for W-S were intermediate between the values obtained for
333 Sk-S and Sd-S, although usually closer to Sk-S. Previous studies have also reported a relative proportion of
334 seeds ranging from 38% to 52% of wine pomace dry material (Teixeira et al., 2014).
335 Wine pomace is rich in phenolic acids (Alonso et al., 2002; Kammerer et al., 2004), which is supported by
336 the considerable concentrations of many of these simple phenolics found in the RWPSs. A greater

337 contribution of hydroxybenzoic than hydroxycinnamic acids to the total of phenolic acids was found,
338 independently of the wine pomace material used for their production, although differences between the three
339 types of RWPSs were detected in the distribution of most of the individual compounds.
340 Catechin, epicatechin, and procyanidins B1 and B2 are also present in significant amounts in wine pomace
341 skins and seeds (Chamorro et al., 2012; González-Paramás, Esteban-Ruano, Santos-Buelga, De Pascual-
342 Teresa, & Rivas-Gonzalo, 2004). These flavan-3-ols were determined in the present study, showing that Sd-
343 S was a richer source of flavan-3-ols than Sk-S. These results were in accordance with previous studies
344 (Kammerer et al., 2004; Monagas et al., 2003; Rockenbach et al., 2011). Nonetheless, the polymeric fraction
345 (oligomers with a higher degree of polymerization and condensed tannins) are known to represent a greater
346 proportion of the total flavan-3-ols in wine pomace (Teixeira et al., 2014), both in seeds (75-81%) and in
347 skins (94-98%) (Monagas et al., 2003). Thus, the contribution of total flavan-3-ols to the total phenolic
348 compounds is expected to be higher than the results obtained in the present study. Nonetheless, the
349 differences observed between the RWPSs may be rather similar, as the total proanthocyanidin contents of the
350 RWPSs have been previously determined (García-Lomillo et al., 2014) and good correlations ($p < 0.001$)
351 between these results and the sums of monomers ($r = 0.967$), dimers ($r = 0.965$), and total flavan-3-ols (r
352 =0.968) obtained in the current study were detected.
353 In the RWPSs containing wine pomace skins (Sk-S and W-S), flavonol-3-*O*-glycosides were more abundant
354 than the flavonol aglycons. These differences might be even more marked as flavonols such as quercetin-3-
355 *O*-glucuronide or quercetin-3-*O*-glucoside are also present in considerable quantities in the wine pomace
356 skins (Kammerer et al., 2004; Sri Harsha et al., 2013; Teixeira et al., 2014) but were not analysed in this
357 study.
358 Anthocyanins are also found in winery by-products mainly as numerous 3-*O*-glycosides derivatives
359 (Kammerer et al., 2004; Pedroza et al., 2012). Malvidin was the most abundant anthocyanidin detected in the
360 seasonings, especially in Sk-S, which agrees with previous studies describing malvidin-3-*O*-glucoside or
361 malvidin (after acidic hydrolysis) as the predominant compounds in the skins of wine pomace (Kammerer et
362 al., 2004; Pedroza et al., 2012). The results obtained were also well correlated ($r = 0.924$, $p < 0.001$) with the
363 total anthocyanin content in RWPSs reported by García-Lomillo et al., (2014). Although a minor quantity of
364 anthocyanidins was detected in Sd-S compared to Sk-S, their presence in wine pomace seeds might be due to
365 their contact with the skins and pomace during winemaking. These compounds might also come from the
366 remnants of skins left during manual separation of the seeds from wine pomace. Surprisingly, cyanidin was

367 detected in a small but larger amount in Sd-S than in Sk-S, possibly due to release from proanthocyanidins as
368 a consequence of the acidic hydrolysis treatment applied to the extracts.
369 Despite Sk-S contained the highest quantities of total identified phenols and Sd-S the lowest, the high
370 contribution of anthocyanidins had a marked influence on these results. This fact was confirmed by
371 comparing data from the HPLC analysis and the TAC characterization.
372 The FC and ABTS assays are considered among the most suitable methods for evaluating the TAC of
373 foodstuffs in previous works, using both classical (C-) (Prior, Wu, & Schaich, 2005; Rivero-Pérez, Muñiz, &
374 Gonzalez-Sanjosé, 2007) and QUENCHER (Q-) approaches (Del Pino-García et al., 2015), so they were
375 used in the present study.
376 As expected, important differences were observed between the C-TAC and Q-TAC results obtained in each
377 assay (FC or ABTS). However, a positive correlation was detected upon comparing the TAC values for the
378 assays using the same approach (C- or Q-). It must be noted that both compounds soluble in the reaction
379 medium and insoluble antioxidants attached to the RWPS matrices can exert their antioxidant activity in Q-
380 TAC methods, whereas only those compounds soluble in the extraction solvent are detected in C-TAC
381 assays. However, some of the phenolic compounds removed from RWPSs during the extraction procedure
382 may not be accessible under real conditions in the foodstuff or biological systems, where water is usually
383 present in the surrounding medium. As a result, classical protocols might overestimate the TAC of those
384 products containing significant quantities of insoluble antioxidants if these are released from the RWPS
385 matrices during the extraction process. This hypothesis is supported by the lower Q-TAC generally obtained
386 for Sd-S compared with the other RWPSs, whereas this seasoning rich in hydrophobic antioxidants gave the
387 highest C-TAC values. In addition, the Q-TAC results obtained for W-S, especially in the Q-ABTS assay,
388 suggest possible synergistic interactions between compounds from both wine pomace skins and seeds, as
389 described previously in the literature (Arnous, Makris, & Kefalas, 2001; Kanner, Frankel, Granit, German, &
390 Kinsella, 1994; Yang, Martinson, & Liu, 2009). These synergisms were evident when both soluble and
391 insoluble antioxidants attached to the RWPS matrices were present in the reaction (Q-TAC methods) but not
392 when RWPS extracts were used (C-TAC essays). In agreement with this finding, the possible regeneration of
393 antioxidants bound to insoluble food matter by soluble antioxidant compounds must be considered, as
394 recently discussed by Çelic et al., (2013).
395 Numerous strong correlations were obtained among the TAC and the HPLC-based total phenolic content
396 values. C-TAC assays were only positively correlated with total flavan-3-ols as Sd-S also contained the

397 highest quantity of these compounds. This finding was consistent with previous studies (González-Paramás
398 et al., 2004) and suggests that flavan-3-ols may be the main phytochemicals responsible for the C-TAC
399 results for the RWPS extracts. In contrast, flavan-3-ols might have a smaller influence in the Q-TAC assays,
400 particularly in the Q-FC results, which may be primarily determined by compounds with high water
401 solubility and which are easily accessible or extractable from the insoluble RWPS matrices, such as
402 anthocyanidins, stilbenes, and flavonols.
403
404 *4.2. Changes induced by the heat treatment*
405 Different mechanisms have been proposed to explain the changes in phenolic composition of products
406 exposed to high temperatures. Thus, a decrease in phenolic content might be due to the onset of thermal
407 degradation of certain phenolic compounds (Maillard & Berset, 1995; Ross et al., 2011). In this sense, there
408 is a general consensus regarding the loss of anthocyanins upon heating. Sólyom et al., (2014) reported that
409 first-order kinetics can be applied to describe the concentration evolution (dC/dt) of monomeric anthocyanins
410 during heating, although the stability of these compounds may vary considerably depending on the nature,
411 extent, and duration of the high temperatures (Khanal et al., 2010). In the present study, the anthocyanin
412 phenolic class was the most severely affected by heat treatment for all the RWPSs, followed by stilbenes and
413 flavan-3-ols. The negative effect of heating was more marked for epicatechins than for catechins, and for
414 procyanidins B2 (epicatechin-(4β→8)-epicatechin) than for B1 (epicatechin-(4β→8)-catechin). A possible
415 explanation for this lies in the structure of grape proanthocyanidins as, at least in grape seeds, these
416 polymeric compounds mainly comprise catechin as the terminal and epicatechin as the extension subunits,
417 thus meaning that catechin and procyanidin dimer B1 are expected to be the major products of their
418 breakdown (González-Paramás et al., 2004). In contrast, the increase in phenolic acid and flavonol contents
419 of the RWPSs could be explained by the liberation of phenolic compounds bound to the food matrices and
420 the partial degradation of lignin, which leads to the release of phenolic acid derivatives (Maillard & Berset,
421 1995; Pinelo et al., 2006; Ross et al., 2011). In the case of gallic acid, which is one of the most widely
422 studied phenolic acids affected by heat treatment, its increase in heat-treated products may also be due to the
423 release of gallate groups from flavan-3-ol gallates (Chamorro et al., 2012; Davidov-Pardo et al., 2011).
424 Moreover, heat treatment seems to also induce a breakage of some esterified forms of hydroxycinnamic
425 acids such as coutaric and fertaric acids, especially notable in Sk-S and W-S, thus increasing their contents in
426 coumaric and ferulic acids.

427 The matrix structures of wine pomace constituents and the interactions of their phytochemicals with such
428 matrices are both aspects affected by thermal processing (Mohdaly, Ahmed, & Smetanska, 2010) and may
429 explain the different impact of heat treatment on the phenolic contents for each type of RWPS. It should be
430 borne in mind that some wine pomace polyphenols are free and can be found in vacuoles, whereas others are
431 associated with cell wall compounds or polysaccharide structures in the skin cells (Arranz et al., 2010; Pinelo
432 et al., 2006). Generally, the increases observed in the content of some phenolic compounds upon heat
433 treatment were more marked for Sd-S, whereas the decreases were more pronounced for Sk-S and W-S.
434 Thus, it seems that phenolic compounds are more susceptible to the detrimental effects of heating when
435 embedded in the RWPS matrices derived from seeds than from seedless or whole wine pomace. However, no
436 important differences were detected when considering the main phenolic classes rather than the individual
437 phenolic compounds, except for total flavonols and total phenolic compounds, which showed the above-
438 mentioned RWPS matrix effect.
439 The negative effect of heat treatment determined in the C-TAC assays was less marked than that observed
440 for the total phenolic content detected by HPLC, especially when evaluated using the C-FC assay. One
441 possible explanation for the results obtained may be the easier extraction of HT phenolic compounds not
442 identified in the HPLC analysis from the solid matrices, as well as the likely formation of new phenolic
443 compounds from more complex compounds (such as anthocyanidins or flavan-3-ol monomers and
444 oligomers) upon heating (Chamorro et al., 2012; Khanal et al., 2010; Ross et al., 2011). In fact, the
445 correlations obtained suggest that changes in the flavan-3-ol content after heating might be chiefly
446 responsible for the C-TAC changes observed.
447 Different results can be found in the literature as regards the impact of heating (under similar conditions to
448 those applied to the RWPSs) on the TAC of wine pomace or derived products. Following a classical
449 approach, Larrauri et al., (1997) found that the extractable polyphenols (determined using the FC assay) in
450 wine pomace skins decreased significantly (about 20%) in samples dried at 100 ºC to a moisture content of
451 around 8%. However, Pedroza et al., (2012) determined the total polyphenol index (FC assay) in skins of
452 three wine pomaces and found different effects of heat treatment at 90 ºC (positive, no effect, and negative)
453 depending on the source grape variety. Working with whole wine pomace, Chamorro et al., (2012) found a
454 slight increase in the TAC of samples heated at 100 ºC for 60 min, this increase being more marked when
455 using the ABTS (around 15%) than the FC and ferric reducing/antioxidant power (FRAP) assays (around
456 4%). Similarly, Sólyom et al., (2014) observed a slight increase in the antioxidant capacity determined using

457 the FC and oxygen radical absorbance capacity (ORAC) assays after the first hour of heating whole wine
458 pomace to 100 ºC. In contrast, Ross et al., (2011) reported different effects of heating wine pomace seeds to
459 120 ºC for 90 min, with no significant TAC changes being observed when using the FC and 2,2-diphenyl-1-
460 picrylhydrazyl (DPPH) assays, but minor reductions in the antioxidant capacity being detected using the
461 FRAP assay (about 6%) and much more marked reductions when using the ABTS method (about 40%).
462 As noted in the results of the current study, the Q-TAC approach may better represent the real situation in
463 food or biological systems, where water is the primary medium and many of the phenolic compounds
464 extracted from RWPSs using organic solvents may be unable to exert their antioxidant capacity. According
465 to the Q-ABTS assay, Sk-S was less affected than W-S and Sd-S by heat treatment. This finding prompted us
466 to consider that the possible synergisms between compounds present in the wine pomace (increasing their
467 antiradical activity) might be altered by heating, with the phenolic compounds derived from wine pomace
468 seeds playing a more important role in these interactions, or being more negatively affected, than those from
469 skins.
470
471 **5. Conclusions**
472
473 In light of all the above, this study provides a detail characterization of the phenolic profile and antioxidant
474 properties of products obtained from different wine pomace materials (seedless, whole, seeds). The heat
475 treatment applied to these products resulted in a slight decrease in their total phenolic content and TAC.
476 However, there is little evidence against this thermal processing as different heat-related effects, as well as
477 RWPS matrix effects on the impact of heating, were observed depending on the assay used. The decrease in
478 anthocyanidin (Sk-S and W-S) and flavan-3-ol contents (Sd-S) appear to be the main factors governing the
479 detrimental effect of high temperatures on the phenolic composition of the RWPSs. In contrast, heat
480 treatment was found to positively affect the phenolic acid and flavonol contents. Indeed, heat treatment
481 generally led to the same or more beneficial changes in the phenolic profile and TAC of extracts from Sd-S
482 than from Sk-S or W-S, whereas the opposite trend was observed when TAC was evaluated directly for
483 powdered products using Q-TAC assays. Thus, this study highlights important differences in the TAC results
484 obtained using the classical and QUENCHER approaches, thereby suggesting the need to apply Q-TAC
485 methods for samples that will be used as directly powdered products, rather than as extracts, by the food and
486 nutraceuticals industry.

Conflict of interest

The authors have no conflicts of interest to disclose.

Acknowledgments

The authors are grateful for financial support from the Autonomous Government of Castilla y León through the research project BU282U13. The PhD grants of R. Del Pino-García and J. García-Lomillo (FPU grants AP2010-1933 and FPU12/05494) are funded by the Spanish "Ministerio de Educación, Cultura y Deporte". The authors also thank Dr A. Rodríguez-Mateos (University of Dusseldorf, Germany/University of Reading, UK) for her supervision and assistance during anthocyanidins analyses.

Abbreviations and nomenclature

ABTS, 2,2'-Azinobis 3-ethylbenzothiazoline-6-sulfonic acid, **C-**, classical; **FC**, Folin-Ciocalteu; **NT**, non-treated seasonings; **Q-,** QUENCHER; **Sd-S**, seasoning from wine pomace seeds; **Sk-S**, seasoning from wine seedless pomace; **TAC**, total antioxidant capacity; **HT**, heat-treated seasonings; **W-S,** seasoning from whole wine pomace; **RWPS**, red wine pomace seasoning.

504 **References**

505 Alonso, Á. M., Guillen, D. A., Barroso, C. G., Puertas, B., García, A., Alonso, A. M., … García, A. (2002).
506 Determination of Antioxidant Activity of Wine Byproducts and Its Correlation with Polyphenolic
507 Content. *Journal of Agricultural and Food Chemistry*, *50*(21), 5832–5836.

508 Andersen, Ø. M., & Markham, K. R. (Eds.). (2005). *Flavonoids. Chemistry, Biochemistry and Applications*
509 (CRC Press). Florida: Taylor & Francis.

510 Arnous, A., Makris, D. P., & Kefalas, P. (2001). Effect of Principal Polyphenolic Components in Relation to
511 Antioxidant Characteristics of Aged Red Wines. *Journal of Agricultural and Food Chemistry*, *49*(12),
512 5736–5742.

513 Arranz, S., Silván, J. M., & Saura-Calixto, F. (2010). Nonextractable polyphenols, usually ignored, are the
514 major part of dietary polyphenols: a study on the Spanish diet. *Molecular Nutrition and Food Research*,
515 *54*(11), 1646–1658.

516 Arvanitoyannis, I. S., Ladas, D., & Mavromatis, A. (2006). Potential uses and applications of treated wine
517 waste: a review. *International Journal of Food Science and Technology*, *41*(5), 475–487.

518 Çelic, E. E., Gökmen, V., & Fogliano, V. (2013). Soluble antioxidant compounds regenerate the antioxidants
519 bound to insoluble parts of foods. *Journal of Agricultural and Food Chemistry*, *61*, 10329–10334.

520 Chamorro, S., Goñi, I., Viveros, A., Hervert-Hernández, D., & Brenes, A. (2012). Changes in polyphenolic
521 content and antioxidant activity after thermal treatments of grape seed extract and grape pomace..
522 *European Food Research and Technology*, *234*(1), 147–155.

523 Davidov-Pardo, G., Arozarena, I., & Marín-Arroyo, M. R. (2011). Kinetics of Thermal Modifications in a
524 Grape Seed Extract. *Journal of Agricultural and Food Chemistry*, *59*, 7211–7217.

525 Del Pino-García, R., García-Lomillo, J., Rivero-Pérez, M. D., González-Sanjosé, M. L., & Muñiz, P. (2015).
526 Adaptation and Validation of QUick, Easy, New, CHeap, and Reproducible (QUENCHER)
527 Antioxidant Capacity Assays in Model Products Obtained from Residual Wine Pomace. *Journal of*
528 *Agricultural and Food Chemistry*, *63*(31), 6922–6931.

529 Delgado Adámez, J., Gamero Samino, E., Valdés Sánchez, E., & González-Gómez, D. (2012). In vitro
530 estimation of the antibacterial activity and antioxidant capacity of aqueous extracts from grape-seeds
531 (*Vitis vinifera* L.). *Food Control*, *24*(1-2), 136–141.

532 Fontana, A. R., Antoniolli, A., & Bottini, R. (2013). Grape Pomace as a Sustainable Source of Bioactive
533 Compounds: Extraction, Characterization, and Biotechnological Applications of Phenolics. *Journal of*

534 *Agricultural and Food Chemistry*, *61*(38), 8987–9003.

535 García-Lomillo, J., González-Sanjosé, M. L., Del Pino-García, R., Rivero-Pérez, M. D., & Muñiz, P. (2014).
536 Antioxidant and Antimicrobial Properties of Wine By-products and their Potential Uses in the Food
537 Industry. *Journal of Agricultural and Food Chemistry*, *62*(52), 12595–12602.

538 Gökmen, V., Serpen, A., & Fogliano, V. (2009). Direct measurement of the total antioxidant capacity of
539 foods: the “QUENCHER” approach. *Trends in Food Science & Technology*, *20*(6-7), 278–288.

540 González-Paramás, A. M., Esteban-Ruano, S., Santos-Buelga, C., De Pascual-Teresa, S., & Rivas-Gonzalo,
541 J. C. (2004). Flavanol content and antioxidant activity in winery byproducts. *Journal of Agricultural*
542 *and Food Chemistry*, *52*(2), 234–238.

543 González-Sanjosé, M. L., García-Lomillo, J., Del Pino-García, R., Muñiz-Rodríguez, P., & Rivero-Pérez, M.
544 D. (2013). Sazonador de origen vegetal con propiedades conservantes, sustitutivo de la sal, y
545 procedimiento de obtención del mismo. University of Burgos, ES Patent 2524870B2.

546 Kammerer, D., Claus, A., Carle, R., & Schieber, A. (2004). Polyphenol Screening of Pomace from Red and
547 White Grape Varieties (*Vitis vinifera* L.) by HPLC-DAD-MS/MS. *Journal of Agricultural and Food*
548 *Chemistry*, *52*(14), 4360–4367.

549 Kanner, J., Frankel, E., Granit, R., German, B., & Kinsella, J. E. (1994). Natural antioxidants in grapes and
550 wines. *Journal of Agricultural and Food Chemistry*, *42*, 64–69.

551 Khanal, R. C., Howard, L. R., & Prior, R. L. (2010). Effect of heating on the stability of grape and blueberry
552 pomace procyanidins and total anthocyanins. *Food Research International*, *43*(5), 1464–1469.

553 Larrauri, J. A., Rupérez, P., & Saura-Calixto, F. (1997). Effect of Drying Temperature on the Stability of
554 Polyphenols and Antioxidant Activity of Red Grape Pomace Peels. *Journal of Agricultural and Food*
555 *Chemistry*, *45*(4), 1390–1393.

556 Larrauri, J. A., Sanchez-Moreno, C., & Saura-Calixto, F. (1998). Effect of Temperature on the Free Radical
557 Scavenging Capacity of Extracts from Red and White Grape Pomace Peels. *Journal of Agricultural and*
558 *Food Chemistry*, *46*(98), 2694–2697.

559 Louli, V., Ragoussis, N., & Magoulas, K. (2004). Recovery of phenolic antioxidants from wine industry by-
560 products. *Bioresource Technology*, *92*(2), 201–208.

561 Maillard, M. N., & Berset, C. (1995). Evolution of antioxidant activity during kilning: role of insoluble
562 bound phenolic acids of barley and malt. *Journal of Agricultural and Food Chemistry*, *43*(7), 1789–
563 1793.

564 Mohdaly, A. A. A., Ahmed, A. R., & Smetanska, I. (2010). Processing Techniques and Their Effects on Fruit
565 Phytochemicals. In F. Klein & G. Möller (Eds.), *Dietary Fiber, Fruit and Vegetable Consumption and*
566 *Health* (pp. 1–49). Hauppauge, NY, USA: Nova Science Publishers, Inc.
567 Monagas, M., Garrido, I., Bartolomé, B., & Gómez-Cordovés, C. (2006). Chemical characterization of
568 commercial dietary ingredients from *Vitis Vinifera* L. *Analytica Chimica Acta*, *563*, 401–410.
569 Monagas, M., Gómez-Cordovés, C., Bartolomé, B., Laureano, O., & Da Silva, J. M. R. (2003). Monomeric,
570 Oligomeric, and Polymeric Flavan-3-ol Composition of Wines and Grapes from *Vitis vinifera* L. Cv.
571 *Graciano* , *Tempranillo* , and *Cabernet Sauvignon*. *Journal of Agricultural and Food Chemistry*,
572 *51*(22), 6475–6481.
573 Monrad, J. K., Howard, L. R., King, J. W., Srinivas, K., & Mauromoustakos, A. (2010). Subcritical solvent
574 extraction of anthocyanins from dried red grape pomace. *Journal of Agricultural and Food Chemistry*,
575 *58*(5), 2862–2868.
576 Palma, M., Piñeiro, Z., & Barroso, C. G. (2001). Stability of phenolic compounds during extraction with
577 superheated solvents. *Journal of Chromatography A*, *921*, 169–174.
578 Pedroza, M. A., Carmona, M., Pardo, F., Salinas, M. R., & Zalacain, A. (2012). Waste grape skins thermal
579 dehydration: potential release of colour, phenolic and aroma compounds into wine. *CyTA - Journal of*
580 *Food*, *10*(3), 225–234.
581 Pérez-Magariño, S., Ortega-Heras, M., & Cano-mozo, E. (2008). Optimization of a Solid-Phase Extraction
582 Method Using Copolymer Sorbents for Isolation of Phenolic Compounds in Red Wines and
583 Quantification by HPLC. *Journal of Agricultural and Food Chemistry*, *58*(24), 11560–11570.
584 Pinelo, M., Arnous, A., & Meyer, A. S. (2006). Upgrading of grape skins: Significance of plant cell-wall
585 structural components and extraction techniques for phenol release. *Trends in Food Science and*
586 *Technology*, *17*, 579–590.
587 Pinelo, M., Tubilar, M., Jerez, M., Sineiro, J., & Nuñez, M. J. (2005). Effect of Solvent, Temperature, and
588 Solvent-to-Solid Ratio on the Total Phenolic Content and Antiradical Activity of Extracts from
589 Different Components of Grape Pomace. *Journal of Agricultural and Food Chemistry*, *53*(6), 2111–
590 2117.
591 Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant
592 capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*,
593 *53*(10), 4290–4302.

594 Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant
595 Activity Applying an Improved ABTS Radical Cation Decolorization Assay. *Free Radical Biology &*
596 *Medicine*, *26*(98), 1231–1237.
597 Rivero-Pérez, M. D., Muñiz, P., & Gonzalez-Sanjosé, M. L. (2007). Antioxidant Profile of Red Wines
598 Evaluated by Total Antioxidant Capacity, Scavenger Activity, and Biomarkers of Oxidative Stress
599 Methodologies. *Journal of Agricultural and Food Chemistry*, *55*, 5476–5483.
600 Rockenbach, I. I., Gonzaga, L. V, Rizelio, V. M., De Sauza Schmidt Gonçalves, A. E., Genovese, M. I., &
601 Fett, R. (2011). Phenolic compounds and antioxidant activity of seed and skin extracts of red grape
602 (*Vitis vinifera* and *Vitis labrusca*) pomace from Brazilian winemaking. *Food Research International*,
603 *44*(4), 897–901.
604 Rodríguez-Mateos, A., Cifuentes-Gómez, T., George, T. W., & Spencer, J. P. E. (2014). Impact of Cooking,
605 Proving, and Baking on the (Poly)phenol content of Wild Blueberry. *Journal of Agricultural and Food*
606 *Chemistry*, *62*(18), 3979–3986.
607 Ross, C. F., Hoye, C., & Fernandez-Plotka, V. C. (2011). Influence of Heating on the Polyphenolic Content
608 and Antioxidant Activity of Grape Seed Flour. *Journal of Food Science*, *76*(6), 884–890.
609 Sacchi, K. L., Bisson, L. F., & Adams, D. O. (2005). A Review of the Effect of Winemaking Techniques on
610 Phenolic extraction in Red Wines. *American Journal of Enology and Viticulture*, *56*(3), 197–206.
611 Saura-calixto, F. (1998). Antioxidant Dietary Fiber Product : A New Concept and a Potential Food
612 Ingredient. *Journal of Agricultural and Food Chemistry*, *46*(10), 4303–4306.
613 Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-
614 phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16*(3), 144–158.
615 Sólyom, K., Solá, R., Cocero, M. J., & Mato, R. B. (2014). Thermal degradation of grape marc polyphenols.
616 *Food Chemistry*, *159*, 361–366.
617 Spigno, G., Tramelli, L., & De Faveri, D. M. (2007). Effects of extraction time, temperature and solvent on
618 concentration and antioxidant activity of grape marc phenolics. *Journal of Food Engineering*, *81*(1),
619 200–208.
620 Sri Harsha, P. S. C., Gardana, C., Simonetti, P., Spigno, G., & Lavelli, V. (2013). Characterization of
621 phenolics, *in vitro* reducing capacity and anti-glycation activity of red grape skins recovered from
622 winemaking by-products. *Bioresource Technology*, *140*, 263–268.
623 Teixeira, A., Baenas, N., Dominguez-Perles, R., Barros, A., Rosa, E., Moreno, D. A., & Garcia-Viguera, C.

624 (2014). Natural Bioactive Compounds from Winery By-Products as Health Promoters: A Review.
625 *International Journal of Molecular Sciences*, *15*(9), 15638–15678.
626 Volf, I., Ignat, I., Neamtu, M., & Popa, V. I. (2014). Thermal stability, antioxidant activity, and photo-
627 oxidation of natural polyphenols. *Chemical Papers*, *68*(1), 121–129.
628 Yang, J., Martinson, T. E., & Liu, R. H. (2009). Phytochemical profiles and antioxidant activities of wine
629 grapes. *Food Chemistry*, *116*(1), 332–339.
630

Figure captions

Figure 1. Total antioxidant capacity (TAC) for non-treated (NT) and heat-treated (HT) red wine pomace seasonings (RWPSs) (seedless: Sk-S; whole: W-S; seeds: Sd-S) using classical (C-) and QUENCHER (Q-) versions of the FC (Folin-Ciocalteu) and ABTS assays. GAE: gallic acid equivalents; TE: Trolox equivalents. Roman letters: Significant differences (ANOVA, $p < 0.05$) between NT seasonings. Greek letters: Significant differences (ANOVA, $p < 0.05$) between HT seasonings.

Tables

Table 1. Phenolic compound contents (μg/g) in non-treated (NT) and heat-treated (HT) red wine pomace seasonings (RWPSs) (seedless: Sk-S; whole: W-S; seeds: Sd-S).

Phenolic compounds	RWPSs					
	Sk-S		W-S		Sd-S	
	NT	HT	NT	HT	NT	HT
p-OH-benzoic acid	7.73 ± 0.08 c	6.27 ± 0.12 γ	4.53 ± 0.09 b	4.01 ± 0.13 β	0.755 ± 0.165 a	0.523 ± 0.124 α
Salicylic acid	5.88 ± 0.68 a	18.9 ± 0.13 γ	6.15 ± 0.76 a	11.8 ± 0.7 β	7.48 ± 0.47 a	7.72 ± 0.23 α
Protocatechuic acid	15.7 ± 0.07 b	21.3 ± 0.16 γ	14.5 ± 0.4 ab	19.6 ± 0.4 β	14.1 ± 0.08 a	16.6 ± 0.2 α
Gallic acid	62.8 ± 0.4 a	73.1 ± 0.4 α	70.8 ± 1.5 b	97.0 ± 0.4 β	95.1 ± 0.3 c	127 ± 0.1 γ
Vanillic acid	27.3 ± 0.2 c	30.4 ± 0.3 γ	17.2 ± 0.13 b	20.3 ± 0.3 β	7.19 ± 0.19 a	9.43 ± 0.08 α
Syringic acid	60.3 ± 0.6 c	74.4 ± 3.5 γ	38.2 ± 1.6 b	48.0 ± 0.8 β	1.56 ± 0.26 a	2.87 ± 0.70 α
Ellagic acid	20.4 ± 0.03 b	21.4 ± 0.06 β	8.88 ± 0.15 a	10.6 ± 0.2 α	ND	ND
Ethyl gallate	19.2 ± 0.6 a	17.1 ± 0.13 α	39.6 ± 1.2 b	33.2 ± 0.9 β	65.0 ± 2.0 c	58.7 ± 2.0 γ
Total Hydroxybenzoic acids	219 ± 0.02 b	263 ± 3 γ	199 ± 5 a	244 ± 2 β	191 ± 2 a	223 ± 1.21 α
p-Coumaric acid	3.00 ± 0.07 a	5.85 ± 0.03 α	5.40 ± 0.32 b	8.78 ± 0.34 β	9.16 ± 0.36 c	9.14 ± 0.58 β
Caffeic acid	2.55 ± 0.42 a	3.40 ± 0.42 α	3.70 ± 0.26 a	4.75 ± 0.21 α	4.97 ± 0.10 b	5.52 ± 0.60 α
Ferulic acid	0.753 ± 0.314 a	1.18 ± 0.01 γ	0.653 ± 0.225 a	0.962 ± 0.037 β	ND	0.270 ± 0.115 α
Coutaric acid	8.99 ± 0.80 c	4.59 ± 0.34 β	5.22 ± 0.21 b	2.42 ± 0.32 α	3.05 ± 0.08 a	2.99 ± 0.02 α
Caftaric acid	13.1 ± 0.3 b	13.6 ± 0.4 β	8.71 ± 1.78 a	10.9 ± 1.3 β	9.44 ± 0.15 a	9.98 ± 0.88 α
Fertaric acid	4.91 ± 0.04 b	3.76 ± 0.25 β	1.99 ± 0.18 a	1.72 ± 0.21 α	ND	ND
Total Hydroxycinnamic acids	33.3 ± 2.0 b	32.4 ± 1.6 α	25.7 ± 2.5 a	29.6 ± 1.7 α	26.6 ± 1.9 a	27.9 ± 1.9 α
Total Phenolic acids	**252** ± 2 **b**	**295** ± 5 **γ**	**226** ± 8 **a**	**274** ± 3 **β**	**217** ± 0.6 **a**	**250** ± 2 **α**
t-resveratrol	2.41 ± 0.07 b	1.73 ± 0.13 α	1.81 ± 0.06 a	1.58 ± 0.014 α	ND	ND
t-piceid	1.88 ± 0.03 b	1.42 ± 0.05 β	1.05 ± 0.06 a	0.645 ± 0.050 α	ND	ND
Total Stilbenes	**4.29** ± 0.10 **b**	**3.16** ± 0.18 **β**	**2.86** ± 0.13 **a**	**2.23** ± 0.04 **α**	**ND**	**ND**

Results expressed as the mean value ± standard deviation (n = 3).

Roman letters: Significant differences (ANOVA, $p < 0.05$) between NT seasonings for each phenolic compound.

Greek letters: Significant differences (ANOVA, $p < 0.05$) between HT seasonings for each phenolic compound.

Table 1. (*Continuation*)

Phenolic compounds	RWPSs					
	Sk-S		W-S		Sd-S	
	NT	HT	NT	HT	NT	HT
Catechin	56.6 ± 1.3 a	47.7 ± 2.0 α	193 ± 2 b	172 ± 1.1 β	389 ± 6 c	380 ± 15 γ
Epicatechin	24.1 ± 2.2 a	20.0 ± 1.6 α	118 ± 3 b	88.0 ± 1.2 β	311 ± 3 c	268 ± 7 γ
Total Flavan-3-ols (monomers)	80.7 ± 0.9 a	67.7 ± 3.6 α	311 ± 6 b	260 ± 0.06 β	701 ± 9 c	649 ± 21 γ
Procyanidin B1	94.8 ± 3.6 a	93.0 ± 4.3 α	107 ± 1.0 b	118 ± 5 β	131 ± 6 c	144 ± 7 γ
Procyanidin B2	60.2 ± 9.0 a	39.2 ± 1.5 α	199 ± 5 b	152 ± 7 β	407 ± 17 c	339 ± 16 γ
Total Flavan-3-ols (dimers)	155 ± 5 a	132 ± 5.8 α	306 ± 4 b	270 ± 2 β	537 ± 11 c	483 ± 9 γ
Total Flavan-3-ols	**236** ± 4 **a**	**200** ± 9 **α**	**618** ± 9 **b**	**530** ± 2 **β**	**1238** ± 20 **c**	**1131** ± 13 **γ**
Kaempferol	3.09 ± 0.06 a	3.58 ± 0.09 α	3.18 ± 0.26 a	4.23 ± 0.107 β	2.87 ± 0.05 a	6.38 ± 0.07 γ
Quercetin	14.6 ± 0.4 a	19.0 ± 0.8 α	16.9 ± 0.16 b	18.5 ± 0.6 α	25.5 ± 0.2 c	22.1 ± 1.3 β
Myricetin	59.2 ± 2.0 c	69.5 ± 2.7 γ	51.3 ± 1.3 b	61.3 ± 1.16 β	20.1 ± 1.3 a	33.0 ± 0.97 α
Total Flavonol aglycones	76.9 ± 1.7 b	92.0 ± 2.0 γ	71.4 ± 0.8 b	84.0 ± 0.7 β	48.4 ± 1.4 a	61.5 ± 0.4 α
Kaempferol-3-O-rutinoside	129 ± 1.1 c	122 ± 2 γ	74.5 ± 0.07 b	77.4 ± 1.0 β	11.5 ± 0.6 a	13.2 ± 0.5 α
Kaempferol-3-O-glucoside	55.2 ± 0.4 c	51.6 ± 0.4 γ	34.8 ± 2.2 b	35.0 ± 0.4 β	14.2 ± 0.07 a	14.2 ± 0.11 α
Quercetin-3-O-rutinoside	26.0 ± 1.8 c	24.9 ± 0.4 γ	20.9 ± 0.7 b	19.9 ± 0.7 β	11.5 ± 0.6 a	11.2 ± 0.14 α
Myricetin-3-O-rhamnoside	53.0 ± 0.9 c	52.7 ± 2.5 γ	31.0 ± 2.3 b	35.4 ± 1.9 β	8.58 ± 0.32 a	11.0 ± 0.18 α
Total Flavonol-3-O-glycosides	263 ± 0.07 c	251 ± 5 γ	161 ± 4 b	168 ± 0.2 β	45.7 ± 0.4 a	49.5 ± 0.6 α
Total Flavonols	**340** ± 2 **c**	**343** ± 3 **γ**	**233** ± 5 **b**	**252** ± 0.8 **β**	**94.2** ± 0.99 **a**	**112** ± 1.0 **α**
Delphinidin	428 ± 21 c	301 ± 8 γ	280 ± 2 b	187 ± 10 β	18.6 ± 0.7 a	11.3 ± 0.6 α
Cyanidin	12.1 ± 1.7 a	6.30 ± 1.06 α	21.2 ± 5.4 a	9.47 ± 1.65 α	41.4 ± 1.5 b	35.3 ± 1.5 β
Petunidin	273 ± 13 c	118 ± 20 β	190 ± 4 b	88.7 ± 6.6 β	9.50 ± 0.39 a	8.71 ± 0.74 α
Peonidin	66.8 ± 0.3 c	30.9 ± 2.8 β	54.5 ± 1.0 b	30.2 ± 3.9 β	0.598 ± 0.139 a	0.192 ± 0.090 α
Malvidin	2452 ± 20 c	1577 ± 109 γ	1819 ± 17 b	1108 ± 58 β	78.7 ± 0.5 a	50.1 ± 5.1 α
Total Anthocyanidins	**3231** ± 48 **c**	**2034** ± 135 **γ**	**2366** ± 26 **b**	**1424** ± 43 **β**	**149** ± 0.8 **a**	**106** ± 3 **α**
Total	**4085** ± 14 **c**	**2892** ± 124 **γ**	**3484** ± 13 **b**	**2515** ± 49 **β**	**1764** ± 19	**1657** ± 7 **α**

Results expressed as the mean value ± standard deviation (n = 3).

Roman letters: Significant differences (ANOVA, $p < 0.05$) between NT seasonings for each phenolic compound.

Greek letters: Significant differences (ANOVA, $p < 0.05$) between HT seasonings for each phenolic compound.

Table 2. Percentage (%) changes in the phenolic compound contents of heat-treated (HT) red wine pomace seasonings (RWPSs) with respect to their non-treated (NT) counterparts (seedless: Sk-S; whole: W-S; seeds: Sd-S).

Phenolic compounds	RWPSs		
	Sk-S	W-S	Sd-S
p-OH-benzoic acid	-18.9 ± 1.5 ** a	-11.5 ± 2.7 * a	-30.7 ± 16.5 a
Salicylic acid	221 ± 10 *** c	91.1 ± 11 * b	3.18 ± 3.01 a
Protocatechuic acid	35.5 ± 1.04 *** b	34.8 ± 2.6 ** b	17.5 ± 1.7 ** a
Gallic acid	16.3 ± 0.6 ** a	37.0 ± 0.6 ** c	33.8 ± 0.09 *** b
Vanillic acid	11.1 ± 1.1 ** a	17.7 ± 1.5 ** b	31.7 ± 1.1 ** c
Syringic acid	23.3 ± 5.8 * a	25.8 ± 2.2 * a	83.6 ± 44.4 a
Ellagic acid	4.67 ± 0.30 ** a	19.0 ± 2.6 * b	ND
Ethyl gallate	-11.1 ± 0.7 * a	-16.2 ± 2.4 * a	-9.80 ± 3.07 a
Total Hydroxybenzoic acids	19.7 ± 1.7 ** b	22.3 ± 0.8 ** b	16.6 ± 0.7 ** a
p-Coumaric acid	95.1 ± 8.9 ** c	62.6 ± 6.21** b	-0.205 ± 6.371 a
Caffeic acid	33.5 ± 16.7 a	28.1 ± 5.6 * a	11.1 ± 12.1 a
Ferulic acid	57.3 ± 0.6 * b	47.3 ± 5.7 * a	DHT
Coutaric acid	-48.9 ± 3.78 * a	-53.8 ± 6.2 ** a	-1.74 ± 0.64 b
Caftaric acid	3.66 ± 2.69 a	26.3 ± 14.5 a	5.71 ± 9.35 a
Fertaric acid	-23.3 ± 5.0 * a	-13.7 ± 10.4 a	ND
Total Hydroxycinnamic acids	-2.77 ± 4.89 a	15.3 ± 6.72 a	4.84 ± 0.64 a
Total Phenolic acids	**16.8** ± 2.1 ** a	**21.5** ± 1.5 * b	**15.2** ± 0.7 ** a
t-resveratrol	-28.2 ± 5.4 * a	-12.7 ± 0.8 * a	ND
t-piceid	-24.4 ± 2.5 ** a	-38.4 ± 4.8 * a	ND
Total Stilbenes	**-26.5** ± 4.1 * a	**-22.1** ± 1.3 * a	**ND**

Results expressed as the mean value ± standard deviation (n = 3).
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$: Significance (Student's t test) of the % change in contents of HT with respect to NT for each RWPS.
Roman letters: Significant differences (ANOVA, $p < 0.05$) between Sk-S, W-S, and Sd-S.
ND: Not detected; DHT: Detected only in heat-treated RWPSs.

Table 2. (*Continuation*)

Phenolic compounds	RWPSs		
	Sk-S	W-S	Sd-S
Catechin	-15.7 ± 3.5 * a	-10.9 ± 0.6 ** ab	-2.36 ± 3.77 b
Epicatechin	-17.0 ± 6.8 a	-25.5 ± 0.98 ** a	-13.8 ± 2.2 * a
Total Flavan-3-ols (monomers)	-16.1 ± 4.5 * a	-16.4 ± 0.02 ** a	-7.43 ± 3.07 a
Procyanidin B1	-1.90 ± 4.5 a	10.3 ± 4.6 a	10.1 ± 5.3 a
Procyanidin B2	-34.8 ± 2.6 * a	-23.8 ± 3.6 * b	-16.7 ± 3.9 b
Total Flavan-3-ols (dimers)	-14.7 ± 3.8 a	-11.8 ± 0.7 ** a	-10.2 ± 1.7 * a
Total Flavan-3-ols	**-15.2** ± 4.0 * a	**-14.2** ± 0.3 ** a	**-8.63** ± 1.02 * a
Kaempferol	15.7 ± 2.9 * a	32.9 ± 3.4 * b	122 ± 3 *** c
Quercetin	30.1 ± 5.4 * c	9.55 ± 3.57 b	-13.3 ± 5 a
Myricetin	17.4 ± 4.5 * a	19.4 ± 2.3 * a	64.7 ± 4.8 ** b
Total Flavonol aglycones	19.7 ± 2.6 * a	17.7 ± 0.9 ** a	27.0 ± 0.8 ** b
Kaempferol-3-O-rutinoside	-5.60 ± 1.41 * a	3.83 ± 1.39 b	14.5 ± 4.2 c
Kaempferol-3-O-glucoside	-6.57 ± 0.71 * a	0.640 ± 1.204 b	0.040 ± 0.778 b
Quercetin-3-O-rutinoside	-4.27 ± 1.58 a	-5.08 ± 3.19 a	-2.44 ± 1.20 a
Myricetin-3-O-rhamnoside	-0.453 ± 4.791 a	14.30 ± 6.27 ab	28.0 ± 2.1 * b
Total Flavonol-3-O-glycosides	-4.63 ± 1.96 a	4.00 ± 0.114 * b	8.29 ± 1.37 * b
Total Flavonols	**0.872** ± 0.940 a	**8.20** ± 0.37 * b	**17.9** ± 1.1 ** c
Delphinidin	-29.7 ± 1.8 * b	-33.2 ± 3.6 ** ab	-39.1 ± 3.0 ** a
Cyanidin	-47.9 ± 8.8 a	-55.3 ± 7.8 a	-14.9 ± 3.7 b
Petunidin	-56.4 ± 7.3 * a	-53.4 ± 3.5 * a	-8.33 ± 7.82 b
Peonidin	-53.8 ± 4.2 ** a	-44.6 ± 7.1 * a	-67.9 ± 15.0 a
Malvidin	-35.7 ± 4.5 ** a	-39.1 ± 3.2 ** a	-36.4 ± 6.4 * a
Total Anthocyanidins	**-37.1** ± 4.2 ** ab	**-39.8** ± 1.8 ** a	**-29.1** ± 2.2 ** b
Total	**-29.2** ± 3.02 ** a	**-27.8** ± 1.4 ** a	**-6.04** ± 0.39 * b

Results expressed as the mean value ± standard deviation (n = 3).

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$: Significance (Student's t test) of the % change in contents of HT with respect to NT for each RWPS.

Roman letters: Significant differences (ANOVA, $p < 0.05$) between Sk-S, W-S, and Sd-S.

ND: Not detected; DHT: Detected only in heat-treated RWPSs.

Table 3. Percentage (%) changes in the total antioxidant capacity (TAC) of heat-treated (HT) red wine pomace seasonings (RWPSs) with respect to their non-treated (NT) counterparts (seedless: Sk-S; whole: W-S; seeds: Sd-S) determined using classical (C-) and QUENCHER (Q-) versions of the FC (Folin-Ciocalteu) and ABTS assays.

TAC assays	RWPSs		
	Sk-S	W-S	Sd-S
C-FC	-5.57 ± 0.58 ** a	-5.74 ± 1.39 ** a	-2.49 ± 0.80 b
C-ABTS	-16.2 ± 2.2 ** a	-13.7 ± 1.9 ** ab	-12.4 ± 1.3 ** b
Q-FC	-19.5 ± 2.0 *** a	-21.4 ± 2.1 *** a	-18.8 ± 5.8 * a
Q-ABTS	-13.3 ± 2.2 ** b	-28.1 ± 3.0 *** a	-26.9 ± 3.5 ** a

Results expressed as the mean value ± standard deviation (n = 3).

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$: Significance (Student's t test) of the % change in TAC of HT with respect to NT for each RWPS.

Roman letters: Significant differences (ANOVA, $p < 0.05$) between Sk-S, W-S, and Sd-S for each assay.

Table 4. Correlation analyses.

A) Correlations using TAC values and main phenolic class contents.

	C-FC	C-ABTS	Q-FC	Q-ABTS
C-FC		0.978***	-0.640**	-
C-ABTS			-0.504*	-
Q-FC				0.745***
Total Phenolic acids	-0,668**	-0,786***	-	-0,609**
Total Stilbenes	-0,940***	-0,872***	0,837**	-
Total Flavan-3-ols	0,997***	0,975***	-0,672**	-
Total Flavonols	-0,995***	-0,971***	0,666**	-
Total Anthocyanidins	-0,875***	-0,790***	0,887***	-
Total Phenolic compounds	-0,798***	-0,698**	0,913***	-

B) Correlations using % changes of HT with respect to NT seasonings.

	C-FC	C-ABTS	Q-FC	Q-ABTS
C-FC		0.752*	-	-
C-ABTS				-0.713*
Q-FC				-
Total Phenolic acids	-	-	-	-
Total Stilbenes	-	0.955*	-	-0.844*
Total Flavan-3-ols	0.844**	0.736*	-	-
Total Flavonols	0,723*	-	-	-
Total Anthocyanidins	0.690*	-	-	-
Total Phenolic compounds	0.857**	-	-	-

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$: Significance (Student's t test) of the correlation.

A) n = 18 (unless Total Stilbenes: n = 12)

B) n = 9 (unless Total Stilbenes: n = 6)

Figure 1. (Two-column figure)