

17 **Abstract**

18 Aim of the research was to study the influence of grape (*Vitis vinifera*) pomace powder, a by-
19 product of wine manufacturing, on chemical composition, nutritional properties and physical
20 characteristics of cakes prepared replacing bread wheat flour with 4%, 6%, 8% and 10% grape
21 pomace powder. The addition of growing quantities of grape pomace powder gradually
22 increased ash, lipid, proteins, fibres, free phenolics, anthocyanins and total polyphenol content
23 as well as antioxidant capacity (DPPH, FRAP), while decreased moisture and pH. The main
24 phenolics provided by grape pomace were catechin, gallic acid, quercetin, protocatechuic acid,
25 kaempferol and apigenin. The phenolic acids and flavonoids content increased from 4.1 mg/kg
26 DM (control) to 26.4-60.9 mg/kg DM (cake with 4%-10% grape pomace powder). The colour
27 coordinates L^* and a^* diminished, while b^* augmented. The cake containing 4% grape pomace
28 powder showed the best sensory quality. The addition of grape pomace powder significantly
29 improved the content in free phenolics, highly bioavailable, that are scarce in bread wheat, and
30 thus the nutritional value of cakes without penalising their technological and sensorial
31 attributes. Therefore, grape pomace powder utilisation will give foods with nutritionally
32 enhanced properties; additionally, its utilisation will alleviate the ecological problems
33 connected to its disposal.

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35 Key words: anthocyanins; antioxidant capacity; phenolics; sensorial analysis; *Vitis vinifera*.

36

37 *Chemical compounds studied in this article:*

38 Apigenin (PubChem CID: 5280443); Catechin (PubChem CID: 9064); Ellagic acid (PubChem
39 CID: 5281855); Gallic acid (PubChem CID: 370); Kaempferol (PubChem CID: 5280863);
40 Myricetin (PubChem CID: 5281672); Protocatechuic acid (PubChem CID: 72); Quercetin
41 (PubChem CID: 5280343); Tyrosol (PubChem CID: 10393).

42 **1. Introduction**

43 Every year the food industry produces million tons of waste, difficult to dispose and that could
44 be conveniently utilised as source of high-quality protein, fibre, antioxidants, sugars, etc.
45 Consequently, the industries and the researchers are keenly studying the potential and the best
46 ways for the economical exploitation of by-products *per se* or as source of bioactive
47 components for foods, pharmaceutical and cosmetic products, and many other goods.

48 Grape vine (*Vitis vinifera* L.) is widespread all over the world, with a 2016 total surface of 7.5
49 million hectares and a production of 7.8 million tonnes, about 50% destined to wine production
50 (OIV, 2017). The wine industry generates substantial quantities of waste, such as grape marc,
51 discarded clusters and seeds, sediments and lees; in fact, pomace represents about 20–30% of
52 the original grape weight (Dwyer, Hosseinian, & Rod, 2014).

53 Large amounts of pomace are produced every year, and their disposal can have a major impact
54 on the environment; most wineries normally use it as animal feed, transform it into compost, or
55 discard it without pre-treatments, often causing soil pollution and acidification because of the
56 low pH of phenolic compounds, and oxygen depletion in soil and ground waters by tannins and
57 other compounds (Devesa-Rey et al., 2011; Dwyer et al., 2014).

58 Grape pomace is a rich source of bioactive substances, especially polyphenols, proteins, lipids,
59 soluble dietary fibre (SDF), insoluble dietary fibre (IDF) and minerals (Devesa-Rey et al., 2011;
60 Mildner-Szkudlarz, Bajerska, Zawirska-Wojtasiak, & Górecka, 2012; Teixeira et al., 2014; Yu
61 & Ahmedna, 2013). Hence, several innovative ways have been proposed for a sustainable
62 reutilisation of grape pomace (Devesa-Rey et al., 2011). Within the bakery industry, grape by-
63 products have been successfully used as additives in the production of bread (Mildner-
64 Szkudlarz, Zawirska-Wojtasiak, Szwengiel, & Pacyński, 2011), biscuits (Mildner-Szkudlarz et
65 al. 2012), cereal bars, pancakes and noodles (Rosales Soto, Brown, & Ross, 2012). Other
66 promising products are the cakes which, depending on the method used for their preparation,

67 are classified as foam (e.g. sponge cake) or batter (e.g. pound cake) cakes (Wilderjans, Luyts,
68 Brijs, & Delcour, 2013; Pycarelle et al., 2019).

69 To the best of our knowledge, no information is available on the effect of grape pomace on cake
70 properties. Therefore, aim of our research was to evaluate the influence of grape pomace
71 addition on the chemical, nutritional (bioactive substances content), technological and sensorial
72 characteristics of cakes. To this end, bread wheat flour and grape pomace powder (GPP), as
73 well as cakes prepared with growing proportions (0%, 4%, 6%, 8% and 10%) of GPP were
74 analysed.

75

76 **2. Materials and Methods**

77 *2.1. Materials*

78 The bread wheat flour (T-550 quality) used for cake manufacturing was bought from Sofia Mel
79 industry (Sofia, Bulgaria). The grape pomace powder (GPP) was produced from Muscat
80 Hamburg grapes harvested in 2018 in the Razgrad region (43°32'00" N, 26°31'00" E), Centre-
81 North of Bulgaria. After juice removal, the grape pomace was dried for 48 hours at 60°C in a
82 UFE 500 oven (Memmert GmbH, Schwabach, Germany), ground into powder with an IKA
83 MF10 grinder (IKA®-Werke GmbH & Co. KG, Staufen, Germany) and stored at 4 °C until
84 analysis or cakes preparation. The other ingredients were from local shops in Razgrad
85 (Bulgaria).

86 The cakes were produced in laboratory, using the methodology of Velioglu, Güner, Velioglu,
87 and Çelikyurt (2017) with minor changes. Briefly: 220.0 g wheat flour, 5.3 g baking powder,
88 62.5 g margarine and 175.0 g sugar were blended with a high-speed mixer (Stand Mixer ELITE
89 STM-0248, Timetron Bulgaria, Sofia, Bulgaria) for 3 min; two eggs were added, and the stirring
90 continued for 3 min more; afterwards, 100 mL whole milk and 50 mL sunflower oil were added,
91 and the homogenization continued for 2 min. The enriched cakes were prepared by replacing

92 the flour with 4, 6, 8 and 10% GPP; these percentages were chosen considering the existing
93 information on leavened bakery products (Mildner-Szkudlarz et al., 2011). Finally, 5.3 g baking
94 powder were added, and the batter mixed for 3 min. The cakes were formed in 11x2.5 cm
95 moulds, baked at 170 °C for 15 min in a BES351110M oven (AEG, Bulgaria), cooled to room
96 temperature for 30 min and analysed within 24 hours. Five types of cakes were produced:
97 control (100% wheat flour) and enriched with 4%, 6%, 8% and 10%; two sets of 10 cakes were
98 prepared for each flour or mixture.

99

100 *2.2. Methods*

101 *2.2.1. Chemical characteristics*

102 The moisture and ash content of bread wheat flour, GPP and cakes were determined according
103 to methods 6540:1980 and 5984:2002 (International Standard Organisation), the protein content
104 was determined following the modified Lowry method, as described in Mæhre et al. (2018), the
105 lipid concentration was tested according to Soxhlet method 136 (ICC), the fibre content was
106 assessed as in method 32-07.01 (AACC International) and the cakes pH was determined
107 according to method 02-52.01 (AACC International). All the analyses were performed in
108 double.

109

110 *2.2.2. Phenolic composition*

111 The phenolic compounds of grape pomace powder and cakes were extracted as described by
112 Yilmaz, Brandolini, and Hidalgo (2015) with some variations. Briefly, exactly 1.0 g of sample
113 were extracted three times with 15 mL of 80% methanol. The pooled extracts were evaporated
114 under vacuum and nitrogen flux, resuspended in 2 mL 80% methanol solution, filtered with a
115 0.45 µm PTFE membrane and analysed by RP-HPLC following Hidalgo et al. (2019). The
116 analysis was performed using a column Adamas® C18-AQ 5 µm 4.6 mm×250 mm and a

117 precolumn C18 5 μ m 4.6 mm \times 10 mm (Sepachrom SRL, Rho, Italy) thermostated at 30 °C;
118 pump L-2130, column oven L-2300 and Diode Array Detector L2450 (Elite La Chrom, Hitachi,
119 Tokyo, Japan). Gradient elution was performed using acetonitrile (A) and 1% (v/v) formic acid
120 in water (B) mobile phases at flow rate 1.0 mL/min, following the gradient profile: 0–10 min
121 from 10% to 25% A, 10–20 min linear rise up to 60% A, and from 20 to 30 min linear rise up
122 to 70% A, followed by 10 min reverse to initial 10% A with 5 min of equilibration time.
123 For peak quantification, calibration curves were constructed using standards from Sigma-
124 Aldrich (St. Louis, MO, USA) and recorded at 280 nm for catechin (2.0 - 99.2 mg/L),
125 epicatechin (3.2 - 85.0 mg/L), gallic acid (2.2 - 101.6 mg/L), protocatechuic acid (0.8 - 27.3
126 mg/L) and tyrosol (3.9 - 98.2 mg/L); at 320 nm for apigenin (0.8 - 10.0 mg/L); at 360 nm for
127 ellagic acid (1.3 - 19.2 mg/L), kaempferol (0.1 - 9.5 mg/L), myricetin (1.3 - 17.2 mg/L) and
128 quercetin (1.2 - 21.6 mg/L). The calibration curves were linear in the concentration intervals
129 assessed. The identity of the compound was confirmed by congruence of retention times and
130 UV/Vis spectra with those of pure authentic standards. Unidentified peaks were quantified
131 using the calibration curve of the compound with similar absorption spectrum and named as
132 “phenolic derivative”. The analyses were performed twice; the results are expressed as mg/kg
133 on dry matter (DM) basis.

134

135 *2.2.3. Anthocyanins content, total polyphenols content and antioxidant capacity*

136 The total anthocyanins were extracted and measured by a spectrophotometric method (Abdel-
137 Aal & Hucl, 1999) using a V650 spectrophotometer (Jasco, Japan). The results are expressed
138 as mg/kg DM on the basis of the cyanidin 3-glucoside standard calibration curve. The total
139 polyphenolic content (TPC) of bread wheat flour, GPP and cakes was assessed on 80%
140 methanol extracts by the Folin-Ciocalteu method as described in Nakov et al. (2018a) and was
141 expressed as mg gallic acid equivalent (GAE)/g dry matter (DM). The antioxidant capacity was

142 determined with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, according to Nakov et al.
143 (2018a), and by the ferric reducing antioxidant power (FRAP) with the OxiSelect™ Assay Kit
144 (Cell Biolabs, Inc., San Diego, CA, USA). The DPPH and the FRAP results are presented as
145 $\mu\text{mol Trolox equivalents (TE)/g DM}$.

146

147 *2.2.4. Physical characteristics*

148 *2.2.4.1. Pasting properties*

149 The pasting properties of the bread wheat flour and the GPP-enriched mixtures were tested
150 using a Micro Visco-Amylo-Graph (Brabender OHG, Duisburg, Germany). The following
151 parameters were determined: peak viscosity, breakdown, setback and final viscosity (in
152 Brabender units), and pasting temperature (in °C). The measurements were performed in
153 duplicate.

154

155 *2.2.4.2. Dimensions and colour*

156 Mean width and thickness (mm) of the cakes were calculated from five SC. The volume (cm^3)
157 was determined using a Volscan Profiler (Stable Micro Systems Ltd., Godalming, Surrey, UK),
158 on three samples. The colour, in the *CIE L* a* b** system, was scored with a Chroma Meter
159 CR-400 colorimeter (Konica Minolta, Tokyo, Japan) on two sets of five random SC.

160

161 *2.2.4.3. Textural characteristics*

162 The textural properties of the cakes (hardness, springiness, cohesiveness and chewiness) were
163 measured using a TA-XT2 Plus texture analyser (Stable Micro System Ltd., Godalming, Surrey,
164 UK). After removing the upper part of the cake, the crumb texture profile was analysed using
165 a 5x5 cm diameter probe. The initial force employed (5 g) was applied twice, with a 10 seconds

166 delay between the two applications; the probe speed was 5 mm/sec until a 50% deformation of
167 the sample centre.

168

169 *2.2.5. Sensory analysis*

170 The sensory analysis of the five cake types was performed at University of Ruse “Angel
171 Kanchev”, Branch Razgrad (Bulgaria). Twenty trained people participated to the sensory
172 analysis, after providing an informed consent according to the guidelines on Ethics and Food-
173 Related research defined by the European Union (Alfonsi et al., 2012). Appearance, texture,
174 odour, aroma and taste were scored from 1 to 5, where 1 is extreme dislike and 5 is extreme
175 like; an overall quality score was computed as average of the five traits evaluated.

176

177 *2.3. Statistical analysis*

178 One-way analysis of variance (ANOVA) and, when significant, Fisher`s Least Significant
179 Difference test (LSD) at $p < 0.05$ were performed with the softwares XL STAT 2017 (Addinsoft
180 Inc., Long Island City, NY, USA) and Office Excel 2013 (Microsoft, Redmond, WA, USA).
181 Pearson`s linear correlation analysis was performed with the software Statgraphics® Centurion
182 XVI (Statgraphics Technologies Inc., The Plains, VA, USA).

183

184 **3. Results and Discussion**

185 *3.1. Bread wheat flour and grape pomace powder composition*

186 The flour and the GPP results are presented in Table 1. The bread wheat flour and grape pomace
187 powder composition was different for all the characteristics. The GPP had lower humidity than
188 the flour, but its ash, lipid, protein, soluble and insoluble fibre, were far higher. The GPP was
189 rich in free phenolic acids, phenylethanoids and flavonoids (Table 2), not tested in the refined
190 bread wheat flour where they are extremely scarce (Wang, Yao, He, Wang, Liu, & Zhang, 2013).

191 Additionally, bread wheat flour and GPP differed for anthocyanin content (not detectable vs.
192 976±7 mg/kg DM), TPC (1.00±0.17 vs. 53.7±0.2 mg GAE/g DM), DPPH antioxidant capacity
193 (301±2 vs. 3366±2 µmol TE/g DM) and FRAP antioxidant capacity (1448±11 vs. 9692±23
194 µmol TE/g DM).

195 The composition of grape pomace, and hence of retrievable substances, is broadly influenced
196 by grapes cultivar, maturity, climate, soil and processing conditions; nevertheless, the GPP
197 results were within the variation reported by different authors (Antoniolli, Fontana, Piccoli, &
198 Bottini., 2015; García-Lomillo & González-SanJosé, 2016; Llobera & Canellas, 2007; Özkan,
199 Sagdiç, Baydar, & Kurumahmutoglu, 2004; Xu, Burton, Kim, & Sismour, 2013; Yu &
200 Ahmedna, 2013).

201

202 *3.2. Pasting parameters of the mixtures*

203 Table 3 provides an overview of the pasting parameters of the bread wheat flour and of the
204 GPP-enriched mixtures. The ANOVA (not presented) highlighted significant differences
205 ($p \leq 0.05$) among samples for all pasting properties. The peak viscosity, setback, final viscosity
206 and pasting temperature augmented with increasing percentages of GPP. The viscosity increase
207 could be related to the high content in GPP of soluble dietary fibre, particularly pectin (Yu &
208 Ahmedna, 2013) and/or to the abundant lipids of grape seeds, which may interact with other
209 hydrophobic substances (e.g. gluten), thus increasing viscosity and delaying starch
210 gelatinization (Mironeasa Codină, & Mironeasa, 2012; García-Lomillo & González-San José,
211 2016). Accordingly, in bread dough Mironeasa & Codină (2013) observed an increase in peak
212 viscosity (but a decrease in pasting temperature) after replacing the flour with 2% to 6% citrus
213 fibre and attributed it to a quicker starch gelatinization because of the higher water content in
214 the dough samples with greater fibre content. Similarly, in bread wheat enriched with up to 11%
215 apple pomace powder Masoodi, Chauhan, Tyagi, Kumbhar, and Kaur (2001) found a

216 progressive increase in peak viscosity after substituting part of wheat flour with apple pomace
217 and ascribed it to the gelling effect of pomace pectin.

218

219 *3.3. Chemical characteristics of the cakes*

220 The ANOVA (not shown) proved the existence of broad differences among cakes for all traits,
221 as expected based on GPP composition. The results of the proximate composition are shown in
222 Table 1.

223 The moisture content and pH of the control cakes were significantly higher ($p \leq 0.05$) than those
224 of the GPP-enriched cakes and decreased rapidly with increasing GPP addition. The lower pH
225 of the GPP-enriched cakes is probably related to the presence of organic acids in grape pomace
226 (García-Lomillo & González-SanJosé, 2016). On the other hand, the moisture decrease seems
227 directly linked to the inferior humidity of GPP.

228 The ash, lipid, protein and dietary fibre (soluble, insoluble and total) concentrations increased
229 almost linearly ($r=0.95-0.97$) with augmenting percentages of GPP. The ash is constituted by
230 the minerals, which in grape pomace may vary depending on cultivar, soil type, climate and
231 vineyard management (Lachman et al., 2013; García-Lomillo & González-SanJosé, 2016), as
232 well as type and duration of the maceration processes (García-Lomillo & González-San José,
233 2016). Nevertheless, the ash content in food products increases even with the addition of small
234 quantities (2-15%) of grape pomace (Acun & Gül, 2014; Bender et al., 2017; Theagarajan,
235 Malur Narayanaswamy, Dutta, Moses, & Chinnaswamy).

236 The lipids content of the cakes is mainly due to the presence of margarine, sunflower oil and
237 whole milk among the ingredients, but the increase in the GPP-enriched cakes is a consequence
238 of the abundant presence of lipids in grape seeds (Acun & Gül, 2014; García-Lomillo &
239 González-San José, 2016), inasmuch that they are commonly used to manufacture grapeseed
240 oil (Devesa-Rey et al., 2011), rich in unsaturated fatty acids (García-Lomillo & González-San

241 José, 2016). Similarly, Acun & Gül (2014) and Theagarajan et al. (2019) recorded a significant
242 rise of lipids content in their grape pomace-enriched biscuits; however, Bender et al. (2017) in
243 muffins did not notice a similar trend.

244 The higher protein and dietary fibre content of the GPP-enriched cakes in comparison to the
245 100% bread wheat cakes is related both to the use of protein-rich ingredients (eggs and whole
246 milk) in the preparation of the cakes and to a major concentration in grape pomace, especially
247 in the case of the insoluble dietary fibre. Accordingly, Acun & Gül (2014) and Theagarajan et
248 al. (2019) found that by raising the percentage of grape pomace, the quantity of proteins and
249 total dietary fibre in biscuits increased; similar findings are reported by Bender et l (2017) for
250 muffins. These changes can have significant implications on the technological and textural
251 characteristics of the products (Nakov et al., 2018b): for example, during baking the proteins
252 coagulate, reinforcing the structure of the gluten matrix. The dietary fibre, besides its
253 contribution to the technological quality, has significant implications on several nutritional
254 aspects (Slavin, 2008). The soluble dietary fibre (oligosaccharides, pectin, beta-glucans and
255 gums) is degraded in the colon and finally digested by the bacteria; among other beneficial
256 effects, it helps lowering blood cholesterol and controls blood sugar (Foschia, Peressini,
257 Sensidoni, & Brennan., 2013). The insoluble dietary fibre (cellulose, hemicellulose, lignin,
258 resistant starch) transits intact through the gastrointestinal tract and improves the peristalsis;
259 additionally, is partially fermented in the large intestine and supports the growth of intestinal
260 microflora, including probiotic bacteria (Foschia et al., 2013). Altogether, adequate total dietary
261 fibre intakes contribute to the prevention of diseases like hypertension, diabetes and obesity
262 (Anderson et al., 2009; Foschia et al., 2013).

263

264 *3.4. Phenolic compounds content*

265 The ANOVA for free phenols composition (not presented) showed significant differences
266 ($p < 0.05$) between the control cakes and the samples enriched with GPP. The free phenolics
267 identified in the GPP and in the cakes are reported in detail in Table 2. Catechin and catechin
268 derivative compounds, the most abundant phenols in grape pomace powder (1094.3 mg/kg DM,
269 i.e. 65.8% total), were absent in the control cake. Other flavonoids in GPP were, in decreasing
270 order, epicatechin derivative, apigenin, kaempferol, myricetin and quercetin, while the phenolic
271 acids recovered were protocatechuic acid, gallic acid and ellagic acid, and the only
272 phenylethanoid detected was tyrosol. In the control cake only tyrosol, and in small quantity
273 gallic acid, were found, probably coming from the other ingredients. In the GPP enriched cakes,
274 all the phenolics detected in GPP were found (except ellagic acid and myricetin), with values
275 increasing according to growing GPP concentrations; the only exception was tyrosol, abundant
276 in the control and progressively diminishing in SCs. Hence, the total phenols concentration in
277 the GPP-enriched cakes was significantly superior to the control cake, confirming the
278 usefulness of this winery by-product for the nutritional improvement of bakery products. Even
279 more relevant is that in wheat the bound polyphenols represent the most abundant fraction
280 (77%), followed by conjugated (22%) and free (<0.5-1%) (Li et al., 2008). Bound phenolic
281 acids are highly stable under heat treatments but have poor nutritional significance because of
282 low bioaccessibility; the scarce free form, instead, is the most bioavailable and the least stable
283 (Hidalgo, Brandolini, Čanadanović-Brunet, Četković, & Tumbas Šaponjac, 2018). Therefore,
284 adding grape pomace powder, rich in free phenolics, will significantly improve the polyphenols
285 composition of the cakes.

286 Nevertheless, when the phenolic content of the enriched cakes was compared to the theoretical
287 values computed by mass balance, a relevant loss of these compounds during baking was
288 noticed, going from 31.19 (4% SC) to 39.22 (6% SC) to 44.18 (8% SC) to 49.15 (10% SC).
289 The loss was compound-dependent and showed that the flavonoids were extremely prone to

290 degradation (on average, 61.5%, and similar at all concentrations), while phenolic acids (28.8%,
291 similar across concentrations) and phenylethanoids (from 6.3 at 4% to 28.1 at 10% GPP) had
292 better resistance to enzymatic and thermal degradation.

293

294 *3.5. Anthocyanin, total polyphenols content, and antioxidant capacity*

295 Figure 1 depicts the increase in anthocyanins and TPC as well as in antioxidant capacity (DPPH
296 and FRAP tests) from control cakes up to 10% GPP-enriched SC. The ANOVA (not shown)
297 highlighted the existence of significant differences among the cakes for all these traits. The
298 anthocyanins (Figure 1A) were absent in the control and increased quickly with the grape
299 pomace powder enrichment, reaching 26.4 g/kg DM at 10% GPP. Similarly, the control
300 contained 19.56 ± 1.30 mg GAE/100 g DM TPC (Figure 1B), but the addition of GPP rapidly
301 increased that value to a maximum of 53.73 ± 0.75 mg GAE/100 g DM in cakes with 10% GPP.
302 Anthocyanins are abundant in red grapes, and therefore their increase in GPP-enriched products
303 is not a surprise, as reported also by Theagarajan et al. (2019) for cookies.

304 A TPC increase following GPP addition was reported also in cookies by Acun & Gül (2014)
305 (from 0.0 in the control to 75.1 g/kg GAE with 15% GPP), by Pasqualone et al. (2014) (from
306 440 in the control to 629 mg GAE/kg DM with ca 22% grape mark extract) and by Theagarajan
307 et al. (2017) (from 3.41 to 4.03 mg GAE/g with 6% GPP), and by Hayta, et al. (2014) in bread
308 (from 35.4 in the control to 89.4 mg GAE/100 g DM in bread with 10% GPP). Therefore, the
309 enrichment with grape pomace powder should allow to manufacture functional foods with
310 improved nutritional properties. The phenolic substances have well-known biological activities
311 (antioxidant, antimicrobial, etc.) (García-Lomillo & González-SanJosé, 2016); furthermore,
312 they can react with superoxide anions, hydroxyl radicals and lipid peroxy radicals (molecules
313 responsible of lipid oxidation and products rancidity), thus acting as antioxidants and

314 contributing to extend the shelf-life of foods (Fontana et al., 2013; García-Lomillo & González-
315 SanJosé, 2016).

316 In line with the TPC results, the enrichment with GPP led to a significant antioxidant capacity
317 increase from 340 $\mu\text{mol TE/g}$ (DPPH; Figure 1C) and 1610 $\mu\text{mol TE/g}$ (FRAP; Figure 1D) in
318 the control cakes (no GPP added) to 462 $\mu\text{mol TE/g}$ (DPPH) and 2428 $\mu\text{g TE/g DM}$ (FRAP) in
319 the 10% GPP-added SC. Similarly, Acun & Gül (2014), Hayta et al. (2014), Pasqualone et al.
320 (2014) and Theagarajan et al. (2017) found that grape pomace extracts significantly increased
321 the antioxidant capacity of bakery products.

322 *3.6. Physical characteristics*

323 The ANOVA (not shown) highlighted significant differences ($p < 0.05$) for all the traits assessed;
324 Table 5 reports the information about the physical characteristics of the cakes produced without
325 (control) or with 4%, 6%, 8% and 10% GPP. The width did not differ significantly among the
326 samples up to 8% GPP addition and decreased slightly only in the 10%-enriched SC. The
327 thickness, instead, decreased progressively from 34.1 mm (control) to 30.8 mm (10% GPP).
328 Therefore, the volume did not change significantly from 0 to 6% GPP, but diminished sharply
329 afterwards, down to 120.5 mm with 10% GPP. Acun & Gül (2014) did not record changes in
330 width, thickness and spread ratio in biscuits enriched with up to 15% GPP, while Hayta et al.
331 (2014) recorded a small, not significant decrease (from 1508 to 1425 mL) in leavened bread;
332 on the other hand, Walker, Tseng, Cavender, Ross, & Zhao (2014) observed a substantial
333 volume reduction in muffins with up to 20% GPP, and attributed it to the negative influence of
334 GPP fibres which interfere with the dough structure and decrease CO_2 retention. The colour
335 parameters L^* , a^* , b^* varied with the addition of GPP. The control cake was the brightest (L^*
336 = 58.17), followed by the 4%, 6% and 8% GPP-enriched samples, which had similar luminosity,
337 while the 10% GPP-added cakes was the darkest ($L^* = 50.73$). The a^* parameter diminished
338 progressively from control (43.1) to 10% GPP-enriched cakes (29.4), indicating a decrease of

339 redness. On the contrary, the b^* values were alike in the control and the 4% cake and increased
340 in the other three samples, denoting a yellower tinge. These changes should be attributed to the
341 darker colour of the GPP in comparison to the bread wheat flour and is observed also when
342 other types of whole meals are employed. Our findings are in good agreement with the results
343 of Acun & Gül (2014), Bender et al. (2017) and Hayta et al. (2014), who noticed lower L^* and
344 a^* (and lower b^* ; Bender et al., 2017) in cookies, muffins and breadcrumbs, respectively, after
345 adding grape pomace, while Pasqualone et al. (2014) recorded decreasing L^* and b^* , along with
346 increasing a^* , in biscuits enriched with grape pomace extracts. These alternative behaviours are
347 probably attributable to the different grape varieties and processes utilised in GPP preparation.
348 Changes in CIE coordinates are common after the addition of different pomace to bread wheat
349 products; for example, Tumbas Šaponjac et al. (2016) discovered some darkening in biscuits
350 enriched with sour cherry pomace. In our cakes, the anthocyanin pigments present in the GPP
351 played a major part in colour coordinates changes, but a significant effect on luminosity was
352 probably exerted also by baking, because heating leads to the formation of dark Maillard
353 reaction products as a consequence of reducing sugars (e.g. glucose and fructose, abundant in
354 grape pomace) and aminoacids presence. The different cakes are presented in Supplementary
355 Figure 1.

356 Hardness, springiness, cohesiveness and chewiness of the different SCs are shown in Table 4.
357 The addition of GPP increased hardness (from 35.5 to 63.9 N) and chewiness (from 17.2 to 26.7
358 N), but slightly reduced springiness (from 0.84 to 0.79) and cohesiveness (from 0.58 to 0.51).
359 Bender et al. (2017) and Hayta et al. (2014) observed similar changes in muffins and in bread
360 wheats, respectively, enriched with up to 10% GPP. Similarly, Walker et al. (2014) described
361 a reduction in springiness and an increase in firmness in GPP-enriched muffins, and attributed
362 them to the reduced volume and increased density of the samples, due to the higher water
363 absorption capacity of the fibre.

364

365 *3.7. Sensory characteristics*

366 Consumers appreciation of food products is largely based on visual and sensorial impression.
367 Hence, for new products development a sensory analysis is necessary to test the acceptability
368 of foods. Our analysis showed that the cake with 4% GPP got the highest evaluation marks
369 (Figure 2A) for all four the sensory parameters (appearance, texture, taste and aroma), while
370 the cake with 6% GPP was outstanding for texture; the control was pleasing but received
371 slightly lower scores. Hence, the best total evaluation in terms of sensory analysis was achieved
372 by the cakes enriched with 4% GPP (Figure 2B). Our observation that the addition of small
373 quantities (4-6%) of grape pomace to food products improve the nutritional quality and impart
374 better sensorial characteristics corroborates the findings of Acun & Gül (2014), Bender et al.
375 (2017), Theargarajan et al. (2019), Hayta et al. (2014), Rosales Soto et al. (2012), and Walker
376 et al. (2014) in different bakery products.

377

378 **4. Conclusion**

379 Cakes enriched with grape pomace powder have significantly higher ash, lipids, proteins,
380 anthocyanins, polyphenols, dietary fibre and antioxidant capacity than the control. Particularly
381 important is the increase in free phenolics, highly bioavailable and scarce in bread wheat. The
382 addition of small quantities of grape pomace powder does not worsen the technological
383 characteristics of the cakes and improves their sensory qualities. Therefore, the direct utilisation
384 of grape pomace powder gives foods with nutritionally enhanced properties; additionally, its
385 utilisation in food production will alleviate the ecological problems connected to its disposal.

386

387 **Acknowledgements**

388 The authors thank Emanuele Fagetti for assistance in the chemical analyses.

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532 **Captions to Figures.**

533

534 **Figure 1.** Anthocyanin content (A), total polyphenol content (TPC) (B) and antioxidant
535 capacity (C: DPPH and D: FRAP) of five sponge cakes prepared with increasing quantities
536 (0%, 4%, 6%, 8% and 10%) of grape pomace powder. Error bars represent the standard
537 deviation. Columns with different letters are significantly different ($p < 0.05$) following Fisher`s
538 LSD test.

539

540 **Figure 2.** Sensory characteristics (A) and overall sensorial quality (B) of five sponge cakes
541 sponge cakes prepared with increasing quantities (0%, 4%, 6%, 8% and 10%) of grape pomace
542 powder. Scale from 1 (extreme dislike) to 5 (extreme like). Columns with different letters are
543 significantly different ($p < 0.05$) following Fisher`s LSD test.

544

545 **Supplementary Figure 1.** Photographs of five cakes prepared with increasing quantities (0%,
546 4%, 6%, 8% and 10%) of grape pomace powder