Antioxidant properties and heat damage of water biscuits enriched with sprouted wheat and barley

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Abstract

The germination process, fostered by renewed metabolic activities, leads to changes in many characteristics of cereal kernels that improve their nutritional value. Aim of this study was to evaluate protein, ash, sugars, heat damage (furosine, hydroxymethylfurfural, glucosylisomaltol), carotenoids, tocols, phenols and antioxidant activity (FRAP, ABTS, DPPH, reducing power, superoxide anion, β-carotene bleaching tests) of water biscuits enriched (0, 5, 10 and 20%) with sprouted meals of bread wheat or barley. The meals from sprouted wheat and barley showed high concentrations of total carotenoids (82.6 and 119.7 mg/kg, respectively), tocols (53.4 and 88.2 mg/kg), conjugated (368.0 and 564.2 mg/kg) and bound (1811.6 and 3022.0 mg/kg) phenols. The enriched water biscuits had higher carotenoids, tocols and phenols content, heat damage and antioxidant activity than the controls. The greatest increases were recorded for barley-enriched samples. Therefore, the addition of 15%-20% sprouted wheat or 5%-10% sprouted barley allowed to improve the nutritional quality of water biscuits while limiting the heat damage.

Key words. Antioxidant activity; carotenoids; furosine; phenols; tocols.

1. Introduction

Cereals are the staple food of humankind because their edible kernels are rich sources of carbohydrates, proteins, lipids, vitamins and minerals. During germination the activation of dormant enzymes leads to significant changes in biochemical, nutritional and sensory characteristics. The α- and β-amylases rapidly degrade the carbohydrates, with a consequent surge in reducing sugars, while other enzymes degrade cell walls and enhance the availability of internal nutrients (Olaerts & Courtin, 2018). The proteins are hydrolysed, increasing peptides and amino acids availability (Olaerts & Courtin, 2018). Different vitamins, such as tocols, thiamine, riboflavin, folic acid and vitamin C, as well as carotenoids, are biosynthesized to produce the nutrients for seedling growth (Olaerts & Courtin, 2018). The metabolic activities fuel polyphenols biosynthesis (Benincasa et al., 2015), while the degradation of cell walls leads to an increase of readily-available free phenolic acids (Van Hung, Hatcher, & Barker, 2011). The more abundant presence of antioxidants molecules boosts the antioxidant activity (Alvarez-Jubete, Wijngaard, Arendt, & Gallagher, 2010). Sprouting increases phytase activity, leading to phytic acid degradation and raise micronutrients absorption in the gastrointestinal tract (Gupta, Gangoliya, & Singh, 2015; Olaerts & Courtin, 2018).

Controlled germination improves the nutritional composition of the kernels, but the hydrolytic enzymes activity has a negative impact on some technological properties of cereals (e.g. bread wheat leavening), indicating that the process must be carefully controlled to avoid excessive enzymatic hydrolysis (Olaerts & Courtin, 2018). On non-leavened products, the effects of germination on technological characteristics are less important. In pasta making, sprouting decreases endosperm vitreousness and falling number of durum wheat, but spaghetti quality for colour, breakage, cooking losses and consistency was comparable to controls prepared from non-sprouted kernels (Dick, Walsh, & Gilles, 1974). More recently, Fu, Hatcher and Schlichting (2014) studied pasta with up to 50% sprouted durum wheat semolina and found that proteins and colour were unaffected, vitreousness decreased and α-amylase activity increased but gluten index and alveogram parameters showed only minimal changes. After drying at 85 °C, browning was observed exclusively in samples with more

than 35% sprouted semolina, and after cooking differences in rheological properties (less consistency and higher dry matter loss) were perceived only in 100% sprouted semolina spaghetti. In cookies, only minor changes are reported, i.e. an increase in spread and an improvement in top grain along with a darker colour of the crust (An, 2015; Lorenz & Valdano, 1981).

The growing attention of the consumers for foods that not only satisfy the nutritional needs of the organism, but which also aid in the prevention of diseases is fostering the study of new formulations and the marketing of new products. In this context, the utilisation of sprouted cereal kernels in snacks, pasta and bakery products is gaining momentum, because sprouted grains are perceived as healthful ingredients. Therefore, the purpose of this study was to evaluate some nutritional characteristics, viz. protein, ash, sugars, furosine, carotenoids, tocols and phenolic compounds content, and antioxidant activity, of bread wheat water biscuits prepared with the addition of increasing concentrations (0, 5, 10 and 20%) of meals obtained from sprouted wheat or barley kernels.

2. Materials and methods

2.1. Materials

The biscuit-quality bread wheat cv. Bramante was cropped in 2016-17 in the Sant'Angelo Lodigiano fields of CREA. After harvest, the seeds were milled with a lab mill (Bona, Monza, Italy) that separated the flour from the bran and the germ; the flour was stored at -20 °C until analysis and water biscuit manufacturing. The sprouted kernel meals were prepared as detailed by Aborus et al. (2018) from bread wheat cv. 'Simonida' and barley hybrid 'NS565', kindly provided by the Institute of Field and Vegetable Crops (NS seed), Novi Sad, Republic of Serbia; the meals were packaged in plastic bags and stored at -20 ° C until analysis and water biscuit manufacturing.

2.2. Water biscuits preparation

81 The water biscuits were made only with deionized water and flour or with deionized water, freeze-
82 dried sprouted meal (either wheat or barley) and flour, to avoid the interference of other ingredients
83 used in biscuits and cookies (i.e. fat, sugar and skimmed powdered milk). The control water biscuits
84 were prepared from 80 g bread wheat flour, adjusted to 13.5% humidity, and 40 mL deionized water,
85 while in the sprout-enriched water biscuits 5%, 10% and 20% flour was replaced by equal amounts of
86 barley or wheat sprout meal. The deionized water used for each dough was adjusted considering the
87 moisture of the ingredients. Every dough was kneaded for 90 s with a Hobart C-100 electric mixer
88 (National MFG CO, Lincoln, Nebraska, U.S.A.), rolled to a uniform 3.9 mm thinness and cut with a
89 die-cutter (internal diameter 35 mm), obtaining 12 water biscuits. The water biscuits were cooked in
90 a rotating oven (Ovenlab, National MFG CO, Lincoln, Nebraska, U.S.) at 205 °C for 18 min, cooled
91 at room temperature for 30 min and stored at -20 °C under vacuum in plastic bags. Two different sets
92 of water biscuits were prepared. The water biscuits were ground with a laboratory mill (Braun,
93 Germany) just before the analysis.
94
95 *2.3. Methods*
96 Water activity was assessed at 25 °C using an AquaLab laboratory tool (Decagon Devices Inc.,
97 Washington, USA). Dry matter (DM) was determined gravimetrically, according to method 44-15
98 (AACC, 1995); protein content was determined by method 46-10 (AACC, 1995), employing 5.70 as
99 multiplying factor; ash content was scored according to method 08-03 (AACC, 1995); fructose,
100 glucose, maltose and sucrose were assessed by HPLC, following Hidalgo and Brandolini (2011); all
101 these results are reported as g/100 g DM. Furosine was determined by HPLC as described by Hidalgo
102 and Brandolini (2011) and the results are expressed as milligrams furosine/100 g protein.
103 Hydroxymethylfurfural (HMF) and glucosylisomaltol (GLI) were determined following the HPLC
104 method of Rufián-Henares, Delgado-Andrade and Morales (2008) as described by Hidalgo and
105 Brandolini (2011); the results are expressed as mg/kg DM. Carotenoids and tocols were extracted and
106 quantified by NP-HPLC as described by Hidalgo, Brandolini and Pompei (2010), and by Hidalgo and

Brandolini (2010), respectively; the soluble conjugated and the insoluble bound phenols of flour, sprouted meals and water biscuits were extracted and analysed as described by Yilmaz, Brandolini and Hidalgo (2015); the results are reported as mg/kg DM.

The antioxidant activity was assessed following six different methods: ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS), as described by Yilmaz et al. (2015); reducing power according to Oyaizu (1986); superoxide anion following Gironés-Vilaplana et al. (2012), and β-carotene bleaching (BCB) as outlined by Al-Saikhan, Howard and Miller (1995). The tests were performed on 70% methanol extracts, except for the β-carotene bleaching test which was done both on 70% methanol and on hexane extracts. The antioxidant activity was expressed as millimoles of Trolox equivalent (TE) per kilogram DM. All the analyses were performed in duplicate on flour, sprouted meals and water biscuits; different water biscuits were tested.

2.4. Statistical analysis

The data were processed by analysis of variance (ANOVA), considering as factors the different water biscuit types. When significant differences were found ($p \leq 0.05$), Fisher's lowest significant difference (LSD) at 95% significance level was computed. The analyses were performed using the Statgraphics® Centurion XVI statistical program (Statpoint Technologies, Inc., USA). The average values and the standard deviation were calculated using the Excel program (Microsoft® Office Excel 2016).

3. Results and discussion

3.1. Flour and meals

The ANOVA (not presented) performed on the characteristics of the flour and of the sprouted meals highlighted significant differences for all the parameters studied. Table 1 reports the average values

of protein, ash, sugars and furosine for the three types of raw materials (bread wheat flour, sprouted wheat meal and sprouted barley meal) used in water biscuit production.

The characteristics of Bramante flour are in line with the information available in literature (Faridi, Gaines, & Stout, 2000; Hidalgo & Brandolini, 2011; Hidalgo, Brandolini, Čanadanović-Brunet, Ćetković, & Tumbas-Šaponjac, 2018). The sprouted meals had protein and ash content higher than the white flour, but similar to non-sprouted kernels, i.e. 12.9-19.9 g/100 g and 1.49-2.05 g/100 g in wheat (Rakszegi et al., 2008), and 8.1-14.7 g/100 g and 1.8-3.0 g/100 g in barley (Andersson et al., 2008; Jadhav, Lutz, Ghorpade, & Salunkhe, 1998).

The sugars concentration in the flour was very low, as observed also by Hidalgo et al. (2018) and Hidalgo and Brandolini (2011); glucose was the most abundant reducing sugar, followed by maltose, while the non-reducing sucrose was highest in bread wheat meal. The sprouted meals had higher concentrations of all sugars. Glucose was more abundant than in the sprouted wheats and barleys (0.21-0.43 and 0.15-0.53 g/100 g DM, respectively) analysed by Panfil et al. (2014), probably because of different sprouting conditions. Our kernels were exposed to light during their germination, and Ziegler et al. (2016) demonstrated that the α-amylase activity, which degrades the starch to simple fermentable sugars, is strongly increased in wheat seeds germinated under illumination.

The high furosine values observed in sprouted meals (68.1 and 109.8 mg/100 g protein for wheat and barley) are due to the presence of more reducing sugars and amino acids after germination (Lorenz & D'Appolonia, 1980; Ziegler et al., 2016), that stimulate the Maillard reaction and the development of related molecules.

The main antioxidant compounds (carotenoids, tocols and polyphenols) and the antioxidant activity of flour and sprouted meals are presented in Table 2. The bread wheat flour had low carotenoid concentration, in line with literature reports (Hidalgo & Brandolini, 2008; Ziegler et al., 2016), and a total tocols content comparable to the data reported by Hidalgo and Brandolini (2008). The carotenoid content was very high in the sprouted meals of wheat and, even more, of barley. Hence, the carotenoid concentrations of the sprouted meals were in the same range of highly-touted

158 vegetables such as carrot (283 mg/kg fresh weight; Surles, Weng, Simon, & Tanumihardjo, 2004)
159 and spinach (176.6-226.3 mg/kg fresh weight; Kidmose, Knuthsen, Edelenbos, Justesen, &
160 Hegelund, 2001). Lutein was always the predominant pigment, but in both meals (α+β)-carotene
161 content largely exceeded that of the non-sprouted flour. Ziegler et al. (2016) described a limited
162 carotenoid increase (from 1.3 to 2.0 mg/kg DM) in dark-sprouted wheat but observed that
163 illumination favoured carotenoid synthesis (from 1.3 to 9.3 mg/kg DM) along with chlorophyll
164 formation. Light induces the biogenesis of chloroplasts, which accumulate carotenoids as accessory
165 pigments or photoprotective constituents of their photosynthetic protein complexes (Sun et al., 2018).
166 The tocols content of sprouted bread wheat meal was within the variation (53.2-74.9 mg/kg DM)
167 described by Hidalgo, Brandolini, Pompei and Piscozzi (2006) for non-sprouted seeds; the
168 germination spurred the synthesis of γ-tocopherol (10.1 mg/kg DM), while the other homologues
169 were in the range observed by Hidalgo et al. (2006). The tocols content of sprouted barley meal was
170 higher than those (46.2-68.8 and 28.4-41.6 mg/kg DM) reported by Andersson et al. (2008) and
171 Lachman, Hejtmánková, Orsák, Popov and Martinek (2018), respectively, for non-sprouted barley
172 kernels.
173 In wheat three types of phenols are present: soluble free, insoluble conjugated (esterified to sugars
174 and other low molecular weight components) and insoluble bound (linked to cell wall constituents
175 such as polysaccharides, protein, lignin, cutin or suberin). In our analysis we did not survey the free
176 phenols. Aborus et al. (2017) and Aborus et al. (2018) found free phenols in sprouted wheat and
177 barley kernels, but other authors (e.g. Alvarez-Jubete et al., 2010) did not record their presence,
178 maybe because they are scarce, very unstable, rapidly degradable and therefore rarely detectable. The
179 conjugated and bound polyphenols in sprouted wheat meal (Table 2) were significantly more
180 abundant than those of non-sprouted whole bread wheat meal, i.e. 40.2-46.9 and 612-647 mg/kg DM
181 (Brandolini, Castoldi, Plizzari, & Hidalgo, 2013), implying that these compounds are synthesized
182 during germination. An increase in polyphenols content after sprouting was observed by Žilić et al.
183 (2014) and, limited to the free form, by Benincasa et al. (2015). Ferulic acid was the most abundant

184 phenol in both fractions, followed by gentisic acid (conjugated phenols) and salicylic acid (bound
185 phenols). The conjugated and bound phenols in sprouted barley meal were more abundant than in
186 sprouted wheat, as well as above the range reported by Idehen, Tang and Sang (2017) for non-
187 sprouted barley (86-198 DM and 133-523 mg/kg DM) and by Dvořáková et al. (2008) for sprouted
188 barley (46.7-70.2 and 129.1-305.7 mg/kg). Ferulic acid was again the most abundant phenol, at
189 concentrations higher than those described by Andersson et al. (2008) (21.7-42.5 and 104.3-365.4
190 mg/kg DM) and Dvořáková et al. (2008) (33.3-53.5 and 113.1-242.5 mg/kg DM). A flavonoid,
191 kaempferol, was also present in both sprouted meals. Hence, our results demonstrate that during
192 germination some phenolic compounds are synthesized thanks to the activation of specific metabolic
193 pathways.
194 The *in vitro* antioxidant activity is better determined with different tests; thus, to understand the
195 effects of the different hydrophilic (polyphenols and flavonoids) and lipophilic (carotenoids and
196 tocols) compounds in a more comprehensive way, the antioxidant activity was challenged by several
197 methods. All the tests showed that the activity of the sprouted meals was significantly higher than
198 that of the flour (Table 2). The reducing power assay had slightly lower values than FRAP, the other
199 method based on the ferric ion reduction mechanism.
200 FRAP, ABTS, DPPH and reducing power tests indicated that bread wheat flour had limited
201 antioxidant activity, probably because phenols are scarce in refined flour and polar extraction
202 solutions (e.g. methanol:water 70:30) do not recover adequately the lipophilic antioxidants; the
203 superoxide anion and BCB assays showed trends similar to the other tests.
204
205 *3.2. Water biscuits*
206 The ANOVA (not presented) showed that the percentage of enrichment was the main factor
207 influencing furosine content, while both percentage of enrichment and flour/meal type modified
208 HMF, GLI, carotenoids, tocols, conjugated and bound phenols, and antioxidant activity. The
209 interactions, often significant, had a more limited influence.

Fig. 1 depicts the heat damage in the different water biscuits. Some furosine (93.0 mg/100g protein) was present in the control sample despite the low sugar content of the wheat flour, while other intermediate products of the Maillard reaction were absent; a similar result (110 mg furosine/100 g proteins; no HMF and GLI) was reported by Hidalgo et al. (2018) in bread wheat water biscuits. Furosine, HMF and GLI contents increased with augmenting percentages of sprouted meals. The samples enriched with sprouted wheat in general showed a lower thermal damage (furosine: 416.1 mg/100 g proteins; HMF: 1.4 mg/kg DM; GLI: 0.8 mg/kg DM) compared to those enriched with sprouted barley (furosine: 473.1 mg/100 g protein; HMF: 3.3; GLI: 1.3 mg/kg DM).

Fig. 2 depicts the total carotenoids, tocols, conjugated phenols and bound phenols content of the control and of the sprouted meal enriched water biscuits; the detailed phenols composition is reported in Supplementary Table 1. All the compounds increased with the sprouted meals enrichment: a 20% addition raised carotenoid content by 666.7% in the samples with sprouted wheat and by 1666.7% in those with sprouted barley. The (α+β)-carotene had the greatest proportional increase (on average from 0.1 to 5.3 mg/kg DM), followed by lutein (from 1.0 to 9.2 mg/kg DM). The water biscuits with sprouted barley had about twice the carotenoids contents than those with sprouted wheat; the only exception was zeaxanthin, with similar concentrations in both species. The increase in tocols content was limited (22.6%) in those with sprouted wheat and strong (93.5%) in those with sprouted barley. Furthermore, in the latter a new compound (γ-tocotrienol), absent in flour and sprouted barley meal, was formed. The homologues with the greatest increase were α-tocopherol and α-tocotrienol, particularly in water biscuits with sprouted barley. The phenolic compounds increased too: at 20% addition they reached 111.5 and 161.1 mg/kg DM (conjugated compounds in wheat and barley, respectively) as well as 533.9 and 1115.0 mg/kg DM (bound compounds in wheat and barley, respectively). Gentisic and ferulic acids showed the greatest raise among conjugated phenols, ferulic acid and salicylic acid among bound phenols. The flavonoid kaempferol, both in the conjugate and the bound form, increased proportionally to the percentage of enrichment. In general, the samples

235 with sprouted barley meal showed the highest increase in each phenol, except syringic acid;
236 chlorogenic acid was detected only in barley. Overall, therefore, the water biscuits enriched with
237 sprouted barley had the biggest quantities of carotenoids, tocols and phenolic compounds. An
238 increase of most free phenolic acids in breads enriched with different quantities of sprouted wheat
239 was recorded also by Gawli-Dziki, Dziki, Pietrzak and Nowak (2017).
240 A consequence of the carotenoids, tocols and polyphenols increase in water biscuits enriched with
241 sprouted meals was a strong growth in antioxidant activity (Fig. 3). From very low in the control
242 (FRAP: 0.7; ABTS: 1.2; DPPH: not detected; reducing power: 0.4 mmol TE/kg DM), high values
243 were reached both in the sprouted wheat enriched water biscuits (with 20% meal: 3.6, 2.8, 0.9 and 3.4
244 mmol TE/kg DM, respectively) and in those with sprouted barley (with 20% meal: 9.9, 6.1, 1.8 and
245 5.8 mmol TE/kg DM). Similarly, the superoxide anion and β-carotene bleaching methanol and
246 hexane tests showed an increase in antioxidant activity: from 32.9, 12.2 and 40.4 mm TE/kg DM in
247 the control to 48.8, 12.4 and 74.3 mm TE/kg DM for wheat sprouted-meal enriched and to 62.3, 18.4
248 and 96.7 mm TE/kg DM for barley sprouted-meal enriched water biscuits. The samples
249 supplemented with sprouted barley almost always exhibited an antioxidant activity superior to the
250 sprouted wheat enriched water biscuits.
251 During water biscuits manufacturing some loss of antioxidant molecules was observed. The total
252 carotenoids and tocols content of the control was slightly lower (1.2 and 15.4 mg/kg DM) than that of
253 the flour (1.8 and 24.3 mg/kg DM); in contrast, some phenolic compounds (conjugated vanillic acid,
254 ferulic acid and bound salicylic acid) increased, probably because the production steps destabilised
255 their links with some molecules, e.g. arabinose and short arabinoxylan chains (conjugated
256 compounds), cellulose and lignin (bound compounds) (Dvořáková et al., 2008). For the enriched
257 water biscuits, a theoretical degradation was computed considering flour/meals composition and
258 percentage addition. Thus, total carotenoids loss in sprouted wheat enriched samples varied from
259 39% to 49%, and in sprouted barley enriched samples from 16% to 27%; interestingly, β-
260 cryptoxanthin increased during baking, confirming the report of Hidalgo et al. (2010). For tocols, the

theoretical loss in sprouted wheat enriched water biscuits was slightly lower than for carotenoids (35-37%), while in sprouted barley enriched samples the loss varies from 19 to 42%. On the contrary, the total phenolic content increased in both sprouted wheat and sprouted barley enriched water biscuits (16-43% and 61-82%, respectively).

4. Conclusions

The germination favoured the synthesis of carotenoids (particularly lutein and (α+β)-carotene), conjugated and bound phenols and antioxidant activity. In water biscuits, furosine, HMF and GLI grew according to the enrichment percentages, but more with sprouted barley addition. Carotenoids, conjugated and bound phenolic compounds and antioxidant activity were higher in the enriches samples than in the control; this increase was not observed for tocols, except with sprouted barley flour. Water biscuits preparation and baking led to some degradation of antioxidant compounds.

In conclusion, the addition of wheat or barley sprouted seeds meal improves the nutritional quality of bakery products. A 15-20% (sprouted wheat) or 5-10% (sprouted barley) enrichment improve carotenoid, tocol and polyphenol content while maintaining thermal damage low.

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381 **Legends to Figures**

382

383 **Figure 1.** Furosine, hydroxymethylfurfural (HMF) and glycosylisomaltol (GLI) content of biscuits
384 obtained from bread wheat flour, without (Control) and with the addition (5%, 10% and 20%) of
385 sprouted bread wheat meal or sprouted barley meal. Different letters indicate significant difference
386 between the samples according to the LSD test ($p \leq 0.05$).

387

388 **Figure 2.** Total carotenoids, tocols, conjugated phenolics and bound phenolics content of biscuits
389 obtained from bread wheat flour, without (Control) and with the addition (5%, 10% 20%) of
390 sprouted bread wheat meal or sprouted barley meal. Different letters indicate significant difference
391 between samples according to the LSD test ($p \leq 0.05$).

392

393 **Figure 3.** Antioxidant activity following FRAP, ABTS, DPPH, reducing power, superoxide anion,
394 and beta-carotene bleaching (BCB) tests of methanol extracts and BCB of hexane extracts of
395 biscuits obtained from bread wheat flour, without (Control) and with the addition (5%, 10% 20%)
396 of sprouted bread wheat meal or sprouted barley meal. Different letters indicate significant
397 difference between samples according to the LSD test ($p \leq 0.05$).

398

Table 1. Means (± standard deviation) and LSD test results ($p \leq 0.05$) for water activity (a_w), moisture content (g/100 g), protein, ash, sugars (g/100 g DM) and furosine (mg/100 g protein) in bread wheat flour, sprouted bread wheat meal (WS) and sprouted barley meal (BS). For each parameter, different letters indicate significant difference between the raw materials.

	Bread wheat flour	WS	BS
Protein	$8.5^{c} \pm 0.0$	$10.3^{b} \pm 0.0$	$12.1^{a} \pm 0.0$
Ash	$0.5^{c} \pm 0.0$	$1.8^{b} \pm 0.1$	$3.1^{a} \pm 0.0$
Fructose	$0.0^{c} \pm 0.0$	$0.8^{b} \pm 0.0$	$1.7^{a} \pm 0.1$
Glucose	$0.1^{c} \pm 0.0$	$3.5^{b} \pm 0.2$	$7.0^{a} \pm 0.5$
Maltose	$0.2^{b} \pm 0.0$	$2.2^{a} \pm 0.2$	$2.5^{a} \pm 0.0$
Sucrose	$0.4^{c} \pm 0.0$	$2.9^{a} \pm 0.3$	$1.6^{b} \pm 0.1$
Furosine	$6.7^{c} \pm 0.3$	$68.1^{b} \pm 0.1$	$109.8^{a} \pm 3.9$

406 **Table 2.** Means (± standard deviation) and LSD test results ($p \leq 0.05$) for content in carotenoids, tocols, soluble
407 conjugated and insoluble bound phenolic compounds (mg/kg DM), and antioxidant activity following FRAP, ABTS,
408 DPPH, reducing power, superoxide anion, beta-carotene bleaching (BCB) test of methanol extracts and BCB of hexane
409 extract (mmoles TE/kg DM) of bread wheat flour, sprouted bread wheat meal (WS) and sprouted barley meal (BS).
410 Different letters indicate significant difference between raw materials.
411

	Bread wheat flour	WS	BS
Carotenoids			
(α+β)-carotene	$0.1^{b} \pm 0.0$	$39.0^{a} \pm 3.9$	$44.9^{a} \pm 1.1$
β-cryptoxanthin	nd	$0.1^{b} \pm .0.0$	$0.2^{a} \pm 0.0$
Lutein	$1.6^{c} \pm 0.2$	$42.5^{b} \pm 0.4$	$73.1^{a} \pm 3.7$
Zeaxanthin	$0.1^{b} \pm 0.0$	$1.0^{a} \pm 0.0$	$1.5^{a} \pm 0.3$
Total carotenoids	**$1.8^{c} \pm 0.2$**	**$82.6^{b} \pm 3.5$**	**$119.7^{a} \pm 2.8$**
Tocols			
α-tocopherol	$3.5^{c} \pm 0.1$	$10.1^{b} \pm 0.9$	$13.5^{a} \pm 0.9$
α-tocotrienol	$0.5^{b} \pm 0.1$	$4.0^{b} \pm 0.7$	$31.6^{a} \pm 1.9$
β-tocopherol	$3.7^{c} \pm 0.8$	$7.1^{b} \pm 0.7$	$11.3^{a} \pm 0.6$
β-tocotrienol	$16.6^{b} \pm 2.5$	$22.0^{a} \pm 0.0$	nd
γ-tocopherol	nd	$10.1^{b} \pm 0.4$	$25.4^{a} \pm 2.9$
γ-tocotrienol	nd	nd	$6.4^{a} \pm 0.4$
Total tocols	**$24.3^{c} \pm 3.6$**	**$53.4^{b} \pm 2.7$**	**$88.2^{a} \pm 4.9$**
Conjugated phenolic acids			
Chlorogenic	nr	nr	19.4 ± 0.1
Gentisic	$0.0^{c} \pm 0.0$	$110.6^{b} \pm 14.4$	$141.3^{a} \pm 1.4$
Vanillic	$3.8^{b} \pm 0.2$	$48.0^{a} \pm 1.5$	$47.3^{a} \pm 0.1$
Syringic	$0.9^{c} \pm 0.0$	$22.9^{a} \pm 2.3$	$6.5^{b} \pm 0.2$
Coumaric	$0.0^{c} \pm 0.0$	$42.0^{a} \pm 0.7$	$35.5^{b} \pm 2$
Ferulic	$4.2^{c} \pm 0.2$	$114.1^{b} \pm 2.0$	$187.3^{a} \pm 1.2$
Salicylic	$0.0^{c} \pm 0.0$	$25.4^{b} \pm 2.0$	$113.9^{a} \pm 3.7$
Kaempferol	$0.7^{c} \pm 0.1$	$5.1^{b} \pm 0.5$	$13.0^{a} \pm 1.3$
Total conjugated	**$9.6^{c} \pm 0.1$**	**$368.0^{b} \pm 13.2$**	**$564.2^{a} \pm 5.9$**
Bound phenolic acids			
p-hydroxybenzoic	$0.0^{b} \pm 0.0$	$9.1^{a} \pm 0.8$	$10.5^{a} \pm 1.1$
Gentisic	$0.0^{c} \pm 0.0$	$22.0^{b} \pm 0.7$	$40.4^{a} \pm 0.1$
Vanillic	$0.5^{c} \pm 0.1$	$9.0^{b} \pm 0.6$	$13.3^{a} \pm 0.9$
Syringic	$0.0^{c} \pm 0.0$	$5.3^{a} \pm 0.3$	$2.9^{b} \pm 0.2$
Coumaric	$2.2^{c} \pm 0.2$	$21.9^{b} \pm 1.9$	$67.0^{a} \pm 3.3$
Ferulic	$48.1^{c} \pm 2.2$	$1034.4^{b} \pm 79.7$	$1450.9^{a} \pm 137.8$
Salicylic	$23.3^{c} \pm 1.2$	$705.3^{b} \pm 50.9$	$1399.4^{a} \pm 89.2$
Kaempferol	$0.0^{c} \pm 0.0$	$4.6^{b} \pm 1.2$	$37.6^{a} \pm 2.3$
Total bound	**$74.2^{c} \pm 3.5$**	**$1811.6^{b} \pm 130.1$**	**$3022.0^{a} \pm 234.8$**
Antioxidant activity			
FRAP	$0.2^{c} \pm 0.0$	$10.9^{b} \pm 0.1$	$21.4^{a} \pm 0.3$
ABTS	$0.5^{c} \pm 0,1$	$7.9^{b} \pm 2.3$	$23.7^{a} \pm 0.2$
DPPH	$0.0^{c} \pm 0.0$	$2.5^{b} \pm 0.1$	$7.2^{a} \pm 0.5$
Reducing power	$0.2^{c} \pm 0.0$	$4.2^{b} \pm 0.1$	$7.9^{a} \pm 0.1$
Superoxide anion	$57.2^{c} \pm 0.6$	$76.0^{b} \pm 2.0$	$152.8^{a} \pm 3.3$
BCB	$20.1^{b} \pm 2.2$	$29.1^{a} \pm 2.7$	$33.8^{a} \pm 1.3$
BCB (hexane)	$40.6^{c} \pm 4.8$	$84.0^{b} \pm 1.6$	$108.9^{a} \pm 1.1$

412
413 nd, not detected
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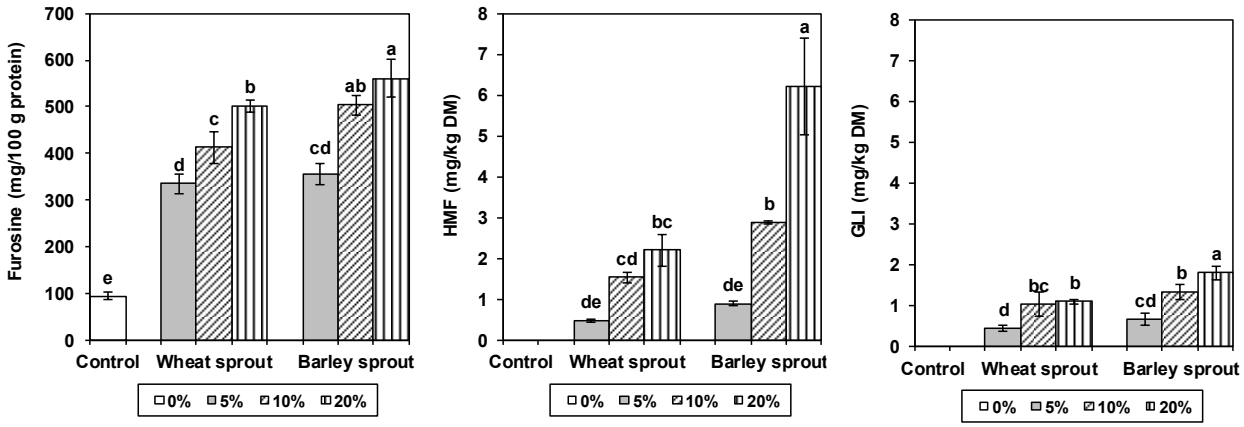

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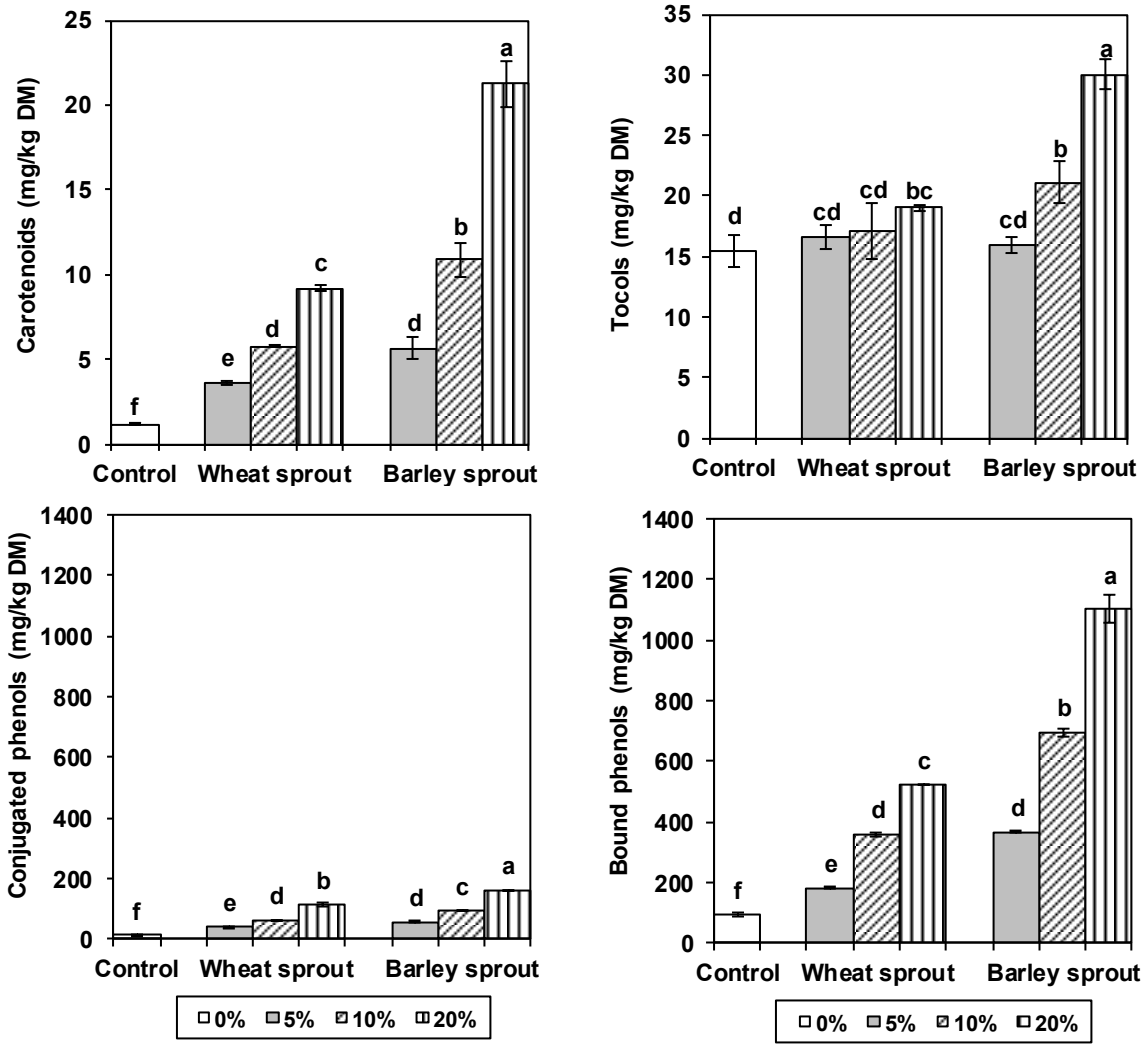
416 Figure 1

417



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419 Figure 2

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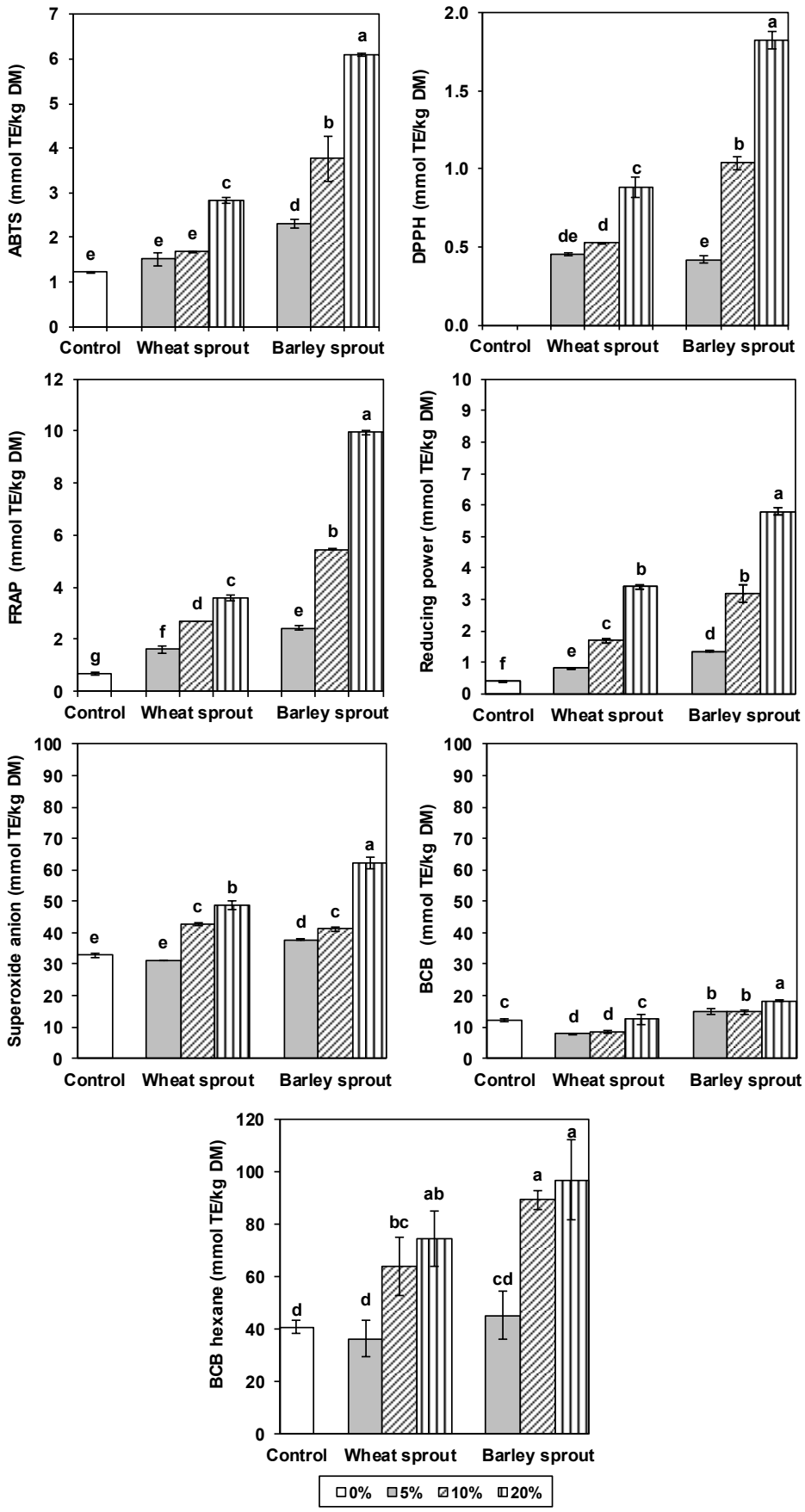

421

422 Figure 3

Supplementary Table 1. Mean (± standard deviation) and LSD test results ($p \leq 0.05$) for content in carotenoids, tocols, soluble conjugated and insoluble bound phenolics (mg/kg DM) in biscuits obtained from bread wheat flour, without (Control) and with addition (5%, 10% and 20%) of sprouted bread wheat meal (WS) or sprouted barley meal (BS). Different letters indicate significant difference between the biscuits.

	Control	WS 5%	WS 10%	WS 20%	BS 5%	BS 10%	BS 20%
Carotenoids							
(α+β)-carotene	$0.1^{f} \pm 0.0$	$0.7^{e} \pm 0.0$	$1.5^{d} \pm 0.1$	$2.8^{c} \pm 0.3$	$1.7^{d} \pm 0.1$	$3.8^{b} \pm 0.3$	$7.7^{a} \pm 0.5$
β -cryptoxanthin	nd	nd	$0.1^{d} \pm 0.0$	$0.1^{c} \pm 0.0$	$0.0^{e} \pm 0.0$	$0.2^{b} \pm 0.0$	$0.4^{a} \pm 0.0$
Lutein	$1.0^{e} \pm 0.1$	$2.7^{d} \pm 0.1$	$4.0^{c} \pm 0.0$	$5.9^{b} \pm 0.1$	$3.8^{cd} \pm 0.5$	$6.6^{b} \pm 0.8$	$12.6^{a} \pm 0.8$
Zeaxanthin	$0.1^{d} \pm 0.0$	$0.2^{bcd} \pm 0.0$	$0.2^{cd} \pm 0.0$	$0.4^{b} \pm 0.1$	$0.2^{d} \pm 0.0$	$0.3^{bc} \pm 0.0$	$0.5^{a} \pm 0.1$
Tocols							
α-tocopherol	$1.5^{f} \pm 0.1$	$2.1^{de} \pm 0.0$	$2.4^{cd} \pm 0.2$	$3.7^{b} \pm 0.5$	$1.7^{ef} \pm 0.1$	$2.8^{c} \pm 0.5$	$4.9^{a} \pm 0.1$
α-tocotrienol	nd	$0.6^{d} \pm 0.2$	$0.9^{d} \pm 0.1$	$1.3^{c} \pm 0.3$	$1.3^{c} \pm 0.0$	$3.1^{b} \pm 0.3$	$6.4^{a} \pm 0.0$
β-tocopherol	2.7 ± 0.4	2.8 ± 0.2	2.7 ± 0.5	2.8 ± 0.2	2.4 ± 0.2	2.7 ± 0.3	3.5 ± 0.5
β-tocotrienol	11.3 ± 0.8	11.2 ± 0.5	11.1 ± 1.4	11.2 ± 0.8	10.0 ± 0.4	10.9 ± 0.5	12.0 ± 0.6
γ-tocopherol	nd	nd	nd	nd	$0.6^{c} \pm 0.1$	$1.1^{b} \pm 0.2$	$2.2^{a} \pm 0.1$
γ-tocotrienol	nd	nd	nd	nd	nd	$0.4^{b} \pm 0.1$	$1.0^{a} \pm 0.0$
Conjugated phenolics							
Chlorogenic	nd	nd	nd	nd	$0.8^{c} \pm 0.3$	$1.5^{b} \pm 0.2$	$3.2^{a} \pm 0.1$
Gentisic	$0.0^{e} \pm 0.0$	$12.6^{d} \pm 2.4$	$19.1^{d} \pm 0.8$	$42.9^{b} \pm 6.6$	$19.4^{d} \pm 1.8$	$30.0^{c} \pm 5.6$	$55.5^{a} \pm 3.8$
Vanillic	$4.9^{d} \pm 0.0$	$7.3^{c} \pm 0.1$	$8.6^{c} \pm 0.2$	$11.9^{ab} \pm 0.2$	$7.8^{c} \pm 0.2$	$10.7^{b} \pm 0.4$	$13.3^{a} \pm 1.5$
Syringic	$1.0^{f} \pm 0.0$	$2.3^{c} \pm 0.1$	$3.8^{b} \pm 0.1$	$6.0^{a} \pm 0.0$	$1.5^{e} \pm 0.2$	$1.9^{d} \pm 0.0$	$2.2^{c} \pm 0.1$
Coumaric	$0.0^{d} \pm 0.0$	$3.4^{c} \pm 0.0$	$6.4^{b} \pm 0.4$	$10.9^{a} \pm 0.4$	$4.2^{c} \pm 0.4$	$6.8^{b} \pm 0.4$	$11.7^{a} \pm 0.7$
Ferulic	$4.4^{f} \pm 0.1$	$10.3^{e} \pm 0.4$	$18.2^{c} \pm 0.8$	$28.9^{b} \pm 0.9$	$14.3^{d} \pm 0.4$	$26.8^{b} \pm 1.3$	$45.2^{a} \pm 2.4$
Salicylic	$0.0^{f} \pm 0.0$	$2.2^{e} \pm 0.5$	$4.2^{d} \pm 0.3$	$7.6^{c} \pm 0.3$	$5.1^{d} \pm 0.0$	$10.8^{b} \pm 1$	$20.8^{a} \pm 0.6$
Kaempferol	$0.6^{e} \pm 0.0$	$1.3^{de} \pm 0.1$	$2.0^{d} \pm 0.3$	$3.3^{c} \pm 0.1$	$3.0^{c} \pm 0.0$	$5.0^{b} \pm 0.2$	$9.2^{a} \pm 0.9$
Bound phenolics							
p-OHbenzoic	$0.0^{e} \pm 0.0$	$5.1^{d} \pm 0.6$	$7.5^{bc} \pm 1.0$	$12.8^{a} \pm 0.2$	$6.6^{cd} \pm 0.1$	$8.8^{b} \pm 1.4$	$13.2^{a} \pm 0.2$
Gentisic	$0.0^{d} \pm 0.0$	$0.0^{d} \pm 0.0$	$2.6^{c} \pm 0.4$	$5.7^{b} \pm 0.7$	$3.0^{c} \pm 0.1$	$6.4^{b} \pm 0.6$	$9.7^{a} \pm 0.9$
Vanillic	$0.6^{e} \pm 0.1$	$0.9^{e} \pm 0.1$	$1.8^{c} \pm 0.2$	$2.4^{b} \pm 0.1$	$1.3^{d} \pm 0.1$	$2.4^{b} \pm 0.1$	$4.3^{a} \pm 0.3$
Syringic	$0.0^{c} \pm 0.0$	$0.0^{c} \pm 0.0$	$1.3^{b} \pm 0.2$	$1.8^{a} \pm 0$	$0.0^{c} \pm 0.0$	$0.0^{c} \pm 0.0$	$1.5^{b} \pm 0.2$
Coumaric	$2.3^{e} \pm 0.3$	$1.0^{de} \pm 0.0$	$3.3^{d} \pm 0.1$	$5.3^{c} \pm 0.0$	$6.0^{c} \pm 0.2$	$13.9^{b} \pm 1.0$	$22.7^{a} \pm 1.5$
Ferulic	$60.9^{f} \pm 2.9$	$117.8^{e} \pm 0.8$	$226.3^{d} \pm 7.1$	$320.5^{c} \pm 2.5$	$213.6^{d} \pm 6.5$	$397.4^{b} \pm 19.6$	$624.2^{a} \pm 0.5$
Salicylic	$28.2^{e} \pm 3.9$	$60.5^{e} \pm 1.9$	$122.3^{d} \pm 0.1$	$182.1^{c} \pm 1.1$	138.7d ± 3.7	$264.2^{b} \pm 7.3$	$423.6^{a} \pm 47.1$
Kaempferol	$0.0^{c} \pm 0.0$	$0.8^{e} \pm 0.1$	$1.8^{d} \pm 0.1$	$3.3^{c} \pm 0.4$	$3.1^{c} \pm 0.4$	$9.1^{b} \pm 0.6$	$15.9^{a} \pm 0.7$

nd, not detected