Phenolic acid composition and antioxidant properties of bran and refined flour from organically and conventionally grown winter wheat

Running title: Healthy properties of milling products from organically cultivated wheat

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ABSTRACT

Since organic foods are supposed to have higher nutritional quality than conventional one, this paper was aimed to study the effects of organic vs conventional cropping system on yield and phenolic composition of winter wheat cv. Bologna. Although organic wheat yielded less than conventional, mainly due to the nitrogen shortage, and its bread-making quality was lower, the cultivation system did not affect the total phenol and total phenolic acid amounts. Moreover, among the 9 phenolic acids recovered, only the ferulic and chlorogenic acids were influenced by the cultivation system. Phenolic composition and amounts were significantly affected by the milling fraction (bran or white flour): phenols were more concentrated in the bran, which showed the highest antioxidant power. In conclusion the adoption of organic cropping system can maintain or even increase the

nutraceutical value of the products from the wheat milling if we agree to accept a reduction in grain yields.

Keywords: organic farming; winter wheat; flour; bran; protein; total phenols; phenolic acids; antioxidant activity; DPPH test; ABTS+ test

1. Introduction

Wheat is the most important Italian arable crop in terms of cultivated area and economic importance, due to the production of bread and pasta that are the main constituents of the Mediterranean diet. In 2011, according to the statistics data (ISTAT, http://agri.istat.it), 1.20 Mha of national arable land were cultivated with durum wheat (*Triticum durum* (Desf.)) and 0.53 Mha to common wheat (*Triticum aestivum* (Linnaeus)), with mean yields of 3.17 and 5.33 Mg ha^{-1}, respectively. In Italy organic farming covers about 8% of the total agricultural land (SINAB, http://sinab.it) and cereals are among the most widespread crops, representing more than 10% of the organically managed land.

The market of organic food in Italy is largely increasing year by year (+8.9% from 2010 to 2011) (ISMEA, http://ismea.it) although the selling prices of organic products are generally higher than those of the conventional ones. The main driver of such consumers' behaviour is likely the highest value recognized to the organic products in terms of environmental sustainability, and also food safety and quality. Actually, organic food is expected by the consumers to be pesticide residues free, more tasty and more healthy than conventional one. However, this assumption has yet to be proved and the relation between the organic production system and the quality of food is far to be fully elucidated.

Many studies focusing on the differences in nutritional values between organic and conventional cereals led to contradictory results (Lairon, 2010; Rembiałkowska, 2007). Generally the organic products show, compared with the conventional ones, a lower

53 content in macronutrients, above all proteins, but also a higher concentration of secondary
54 metabolites. This may be due to the greater exposure of the organic crops to the pest
55 attacks and the nutritional stresses, as a consequence of the limitations on the use of
56 pesticides and chemical fertilizers (Rembiałkowska, 2007).

57 Among the secondary metabolites, phenolic acids received a great attention in the last
58 decade for their beneficial effects on the human health, mostly as preventive agents of
59 chronic diseases, such as obesity, cancer, diabetes, and cardiovascular deficiency. The
60 phenol content in cereals is considered particularly important for the human health, due to
61 their peculiar antioxidant activity and the high rate of cereal foods in the diet of most
62 human population (Slavin, 2004). In wheat, the phenols include derivatives of the benzoic
63 (*p*-hydroxybenzoic, protocatechuic, vanillic, syringic and gallic) and the cinnamic acid (*p*-
64 coumaric, caffeic and ferulic). Ferulic is the dominant phenolic acid in wheat (>90%), as
65 well supported by previous studies (Li, Shewry, & Ward, 2008; Zuchowski, Jonczyk,
66 Pecio, & Oleszek, 2011; Żuchowski, Kapusta, Szajwaj, Jończyk, & Oleszek, 2009). The
67 parent phenolic acids, benzoic and cinnamic acids, have TEAC values of zero,
68 demonstrating no antioxidant activity, whereas the dihydroxylation and the
69 trihydroxylation of the phenol ring seem progressively increase the antioxidant activity of
70 these compounds. In fact, the antioxidant response depends on the relative position of the
71 hydroxyl groups (Sgherri, Cecconami, Pinzino, Navari-Izzo, & Izzo, 2010).

72 In recent years, several studies were carried out on the effects of genotype and
73 agronomic management (i.e. organic or conventional techniques, dates of sowing, different
74 fertilization or crop protection strategies) on the nutritional quality of cereals. Most of
75 them reported a reliable effect of these factors on phenolic contents as well as on their
76 composition in grain and milling fractions, although sometimes the observed differences
77 were not significant (Gasztonyi, Farkas, Berki, Petróczi, & Daood, 2011; Menga, Fares,
78 Troccoli, Cattivelli, & Baiano, 2010; Mpofu, Sapirstein, & Beta, 2006; Stracke, Eitel,

79 Watzl, Mäder, & Rüfer, 2009; Vaher, Matso, Levandi, Helmja, & Kaljurand, 2010;
80 Zuchowski et al., 2009, 2011). The heterogeneity in environmental conditions, both in
81 space (i.e. locations) and time (i.e. weather variability over years) can affect significantly
82 the quantitative and qualitative composition in phenolic compounds.
83 On the other hand, other studies reported very clear differences in phenolic pattern
84 among the different milling products (i.e. grain, refined flour, whole flour, bran) (Adom,
85 Sorrells, & Liu, 2005; Mattila, Pihlava, & Hellstrom, 2005; Vaher et al., 2010). The
86 content of phenolics have been shown to dramatically decrease with increasing level of
87 refinement of flour, whilst it seems to be not affected at all by the baking processes. This is
88 because of the particular distribution of phenols in cereal grain. Kernels contain phenolic
89 acids both in soluble (either free or conjugated) and bound forms. Most of phenols (>90%)
90 is esterified with wall components of cells in the aleuronic layer and pericarps (Naczk &
91 Shahidi, 2006). Thus, the phenols located in those parts of seeds are generally left out
92 during the milling and consequently removed from the refined wheat flour.
93 To our knowledge there have been only few previous studies focusing on the
94 interaction between the cropping system and the wheat products in terms of baking quality
95 and pattern of phenols. This issue seems to be extremely relevant as it could provide
96 information in order to fully express the intrinsic nutritional value of wheat. Thus, the aim
97 of this study was to compare the quali-quantitative composition and the antioxidant
98 activity of the phenolic acids both in the white flour and the bran obtained by milling
99 grains of winter wheat grown under organic and conventional systems. Crop productivity
100 and grain quality for the two management systems have been also taken into account.
101
102 **2. Materials and methods**
103
104 *2.1. Chemicals*

105 All reagents were of the highest purity and were purchased from Sigma-Aldrich
106 (Milan, Italy). Water was of Milli Q grade. All solvents and water were accurately
107 degassed before use.
108
109 *2.2. Site description, agronomic management and sample collection*
110 The present research was performed in 2010 collecting the common wheat samples
111 within the MASCOT (Mediterranean Arable Systems COmparison Trial) long-term
112 research (Mazzoncini, Canali, Giovannetti, Castagnoli, Tittarelli, Antichi, et al., 2009),
113 carried out at the Centre for Agro-Environmental Researches "Enrico Avanzi" of the
114 University of Pisa, located in San Piero a Grado, Italy (43°.40' N; 10°.19' E). The
115 experimental area is about 1 m above mean sea level on a flat land and is characterized by
116 a Typic Xeropsamment loamy soil (44% of sand, 34% of silt and 22% of clay) with a low
117 level of soil organic matter (1.61%) and a pH of 8.4. The climatic conditions are typical of
118 the Mediterranean areas, with about 900 mm of rainfall per year mostly concentrated in
119 autumn (October to December) and in spring (March to April), and with a mean yearly air
120 temperature of 15° C.
121 The research, started in 2001 and still ongoing, compares two rainfed cropping
122 systems, one managed organically (OS) and one conventionally (CS), both subjected to the
123 same 5-year stockless crop rotation: maize (*Zea mais* L.) - durum wheat (*Triticum durum*
124 Desf.) - sunflower (*Helianthus annuus* L.) - pigeon bean (*Vicia faba* L. var. minor) -
125 common wheat (*Triticum aestivum* L.). The five crops in the rotation were allocated to five
126 fields in each block and managed consistently with the system adopted. In this way each
127 group of five fields represents a system within a block and each crop was present every
128 year occupying a whole field to an area of 0.35-1.00 ha. Each system was replicated three
129 times according to a randomized complete block (RCB) design.

130 As regards the wheat cultivation, the differences between organic and conventional
131 management concerned the amount and the type of fertilizers and the methods for weed
132 control. In detail, the fields of the organic system were supplied with 30 U/ha of nitrogen,
133 30 U/ha of P_2O_5 and 30 U/ha of K_2O from Nutex® (cattle manure desiccated and pelleted),
134 whereas the conventional cultivation was conducted by supplying 155 U/ha of nitrogen
135 and 92 U/ha of P_2O_5 from mineral fertilizers (ammonium phosphate and nitrate). The weed
136 control was mechanical in organic system (two passes of flex tine harrow) and chemical in
137 conventional system (post-emergent herbicide treatment applying: iodosulfuron-methyl +
138 mesosulfuron methyl a.i.). Main and secondary tillage were the same for the two cropping
139 systems and consisted on a shallow ploughing (0.25 m deep) and on two harrowing passes
140 (disk and rotary). Also the seeding rate (200 kg ha^{-1} of untreated seed) and the wheat
141 variety (cv. Bologna) were the same. This variety is one of the most used in Italy for the
142 bread production, being characterized by a high quality of the grain (high protein content,
143 considerable test weight and medium-high hardness).
144 From each replicate, at harvest time, 4 samples of 1 m^2 were collected and processed
145 separately to determine the grain yield. Dry matter content of straw, ears and grain of
146 wheat was determined after oven-drying samples at 60°C until constant weight.
147 In order to evaluate the physical, chemical and technological characteristics of the
148 wheat grain, bran and flours, samples of seeds were collected at crop maturity, mixing
149 subsamples from the total pool of grain harvested on each field. Samples, coming from
150 each field, were then kept separated from the others in order to have three different
151 replicates for each cropping system.
152
153 2.3. *Wheat flour preparation*
154 The wheat samples were tempered to a moisture content of 13%. The grain was
155 milled in white flour, with separation of the bran by mean of a mill Buhler (Uzwil,

156 Switzerland) according to AACC Method 26-50 (American Association of Cereal
157 Chemists, 2000). The flour yield was about 80%. The milled flours were kept in a -20°C
158 freezer in airtight containers until analysis.
159
160 *2.4. Technological parameters of grain and flour*
161 The determination of technological quality of whole grain was assessed by
162 measuring wheat hardness (Near Infrared Reflectance spectroscopy, AACC Method 39-
163 70), protein content (N Kjeldahl x 5.74) and SDS (sodium dodecyl sulphate) sedimentation
164 test, useful for assessing the wheat bread-making quality (Preston, March, & Tipples,
165 1982).
166 The characterization of white flour was performed determining the protein content,
167 the water content and the Hagberg Falling Number to have a measure of the amylase
168 activity (ISO3093). Moreover, the two main alveographic parameters were determined: W
169 and P/L, representing the deformation energy of dough (area bounded by the alveogram)
170 and the ratio between tenacity and elasticity of dough (the maximum over pressure needed
171 to blow the dough bubble and the average abscissa at the bubble rupture), respectively.
172
173 *2.5. Extraction of phenolics*
174 The phenols were extracted from the white flour and bran following the method
175 reported by Zuchowski et al. (2009). The samples (0.266 g) were alkaline hydrolyzed
176 under nitrogen with 4N NaOH (1:30 w/v) for 2 hours in the dark. Extraction solution was
177 added with ascorbic acid (1%) and ethylenediaminetetraacetic acid (EDTA, 10 mM) in
178 order to prevent the degradation of phenolic acids during alkaline hydrolysis (Nardini,
179 Cirillo, Natella, Mencarelli, Comisso, & Scaccini, 2002). Then, the samples were acidified
180 using 6 M HCl to achieve a pH value of about 2. The resulting mixtures were extracted
181 three times with 10 mL of ethyl acetate. The organic phase was collected and evaporated to

dryness at 35°C in a rotary evaporator. Immediately before analysis, the residue was re-dissolved in 50% (v/v) acetonitrile (3 ml) and then passed through a Sartorius (Goettingen, Germany) filter (Minisart 0.45 μm) to remove any suspended material.

2.6. Total phenolic content

Determination of the total phenolic compounds was performed on flour extracts following the method reported by Nguyen & Niemeyer (Nguyen & Niemeyer, 2008). Briefly, extract (50 μL), deionized water (450 μL), Folin-Ciocalteu phenol reagent (250 μL), and 20% sodium carbonate (1.25 mL) were added in a test tube, mixed, and allowed to incubate at room temperature for 20 minutes. Absorbance of the samples was then measured at 735 nm, and the calculations were performed using a calibration curve prepared with gallic acid as standard.

2.7. Phenolic acid composition

The qualitative and quantitative analyses of phenolic acids were performed by RP-HPLC following what reported by Sgherri et al. (2010). Twenty microliters of extract were injected into a Waters model 515 HPLC system fitted with a 3.9 mm x 150 mm Nova-Pak C18 column (Waters, Milford, MA, USA). Detection was at 280 nm using a Waters 2487 dual λ UV-VIS detector. Mobile phase A contained 98% water and 2% acetic acid, and mobile phase B contained 68% water, 30% acetonitrile and 2% acetic acid. A linear gradient of 10–95% mobile phase B was run for 70 min at 1 mL min^{-1}. The identity of the phenolic acids was confirmed by co-chromatography on HPLC with authentic standards (Sigma, St. Louis, MO, USA), and quantification was performed using a standard curve in the range of 0.1-0.5 μg of standard mixtures containing gallic (GA), protocatechuic (PCA), *p*-hydroxybenzoic (pHBA), vanillic (VA), chlorogenic (CGA), caffeic (CA), siringic (SA),

207 *p*-coumaric (pCA) and ferulic (FA) acids. Chromatogram analysis was performed by the
208 software Millennium 32 (Waters).
209
210 *2.8. Antioxidant activity*
211 *2.8.1. Radical cation $ABTS^+$ scavenging capacity*
212 The radical cation $ABTS^+$ (2,2'-azino-di-[3-ethylbenzthiazoline sulphonate]) was
213 generated as previously described (Sgherri et al., 2010). The radical solution was diluted in
214 ethanol in order to obtain an absorbance at 734 nm of 0.70 ± 0.05. After addition of the
215 extract, the decrease in absorbance was monitored and compared to that of the Trolox
216 standard. Antioxidant activity after 10 minutes was expressed in terms of mmol Trolox
217 equivalents (TE)/100 g bran or flour.
218
219 *2.8.2. Radical DPPH scavenging capacity*
220 The free radical scavenging capacity of flour extracts was determined using the
221 stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) following what reported by Ragaee,
222 Abdel-Aal and Noaman (2006). The antioxidant reaction was initiated by transferring 1 ml
223 of extract into a test tube containing 4 ml 80% methanol and 1 ml (containing 1 mmol) of
224 freshly prepared DPPH solution. The reaction was monitored by reading absorbance at 517
225 nm. A blank reagent was used to study stability of DPPH over the test time. The
226 absorbance measured at 10 minutes was used for calculation of μmol DPPH scavenged by
227 extracts. Trolox was used as antioxidant reference and antioxidant activity was expressed
228 as mmol TE/100 g bran or flour.
229
230 *2.9. Statistical analysis*
231 Before the statistical analysis of data, the Bartlett's test was performed to verify the
232 homogeneity of error variances. For relative concentration Bartlett's test gave significant

233 results, so a proper transformation of data was applied. The crop yield and white flour
234 parameters were analysed with 1-way analysis of variance (ANOVA) for randomized
235 complete block design with three replications. The differences between treatment means
236 were compared using a Fisher's protected LSD test at $p < 0.05$.
237 The chemical determinations were analysed with a split-plot design, with wheat
238 product (P: white flour and bran) as main plot factor and cropping system (C: organic and
239 conventional,) as subplot factor, in order to assess the possible interactions. The software
240 used for statistical analysis was Cohort Costat (Monterey, CA, USA).
241
242 **3. Results**
243
244 Although in the 2009/10 season the weather was pretty in line with the normal trend for
245 the area, unusual peaks of precipitations occurred in the first two months from sowing, and
246 in the late spring. This peculiar distribution of rainfall caused the nitrate leaching from the
247 soil, and then it could be likely the reason of the yield decrease recorded, which was more
248 severe than usual (data not shown).
249 Table 1 shows that the main biomass parameters at harvest for winter wheat (cv.
250 Bologna) were significantly affected by the cultivation system. In fact, dry matter yield
251 was reduced by about half in organically cultivated winter wheat. However, main quality
252 parameters of grain, such as protein content, were not influenced by the different cropping
253 system (Table 1).
254 For what concerns the properties of the white flour, protein content and W index
255 were significantly lower under the organic system, whilst water content, P/L index and
256 falling number were not affected by the cropping system (Table 2).
257 The total phenols and total phenolic acids were 4-7 fold higher in bran than in white
258 flour (Fig. 1), whatever the cultivation system. Both DPPH and $ABTS^{+}$ test showed that

bran presented an antioxidant activity about twice higher in comparison with white flour (Fig. 2). The organic cultivation did not reduce the total content of phenols and of phenolic acids while induced an increase of antioxidant activity in the bran.

All milling products showed the presence of seven phenolic acids: GA, PCA, *p*-HBA, VA, SA, *p*-CA and FA, whereas CGA and CA could be detected only in the bran (Table 3). The composition in phenolic acids differed in the two wheat products. The main component in wheat flour was represented by SA, which approached 60 and 80% of the total under CS and OS, respectively. In the bran the main phenolic acid was FA, which reached the 60% and the 49% of the total amount of phenolics in CS and OS, respectively (Table 3). In the white flour the presence of FA, PCA and GA was also relevant, whereas VA and *p*-CA were detected at a very low level.

In the bran from conventional system SA and PCA represented, respectively, 19% and 14% of total phenolics. GA and *p*-CA approached 5.92 and 3.52 μmol/100 g, respectively, whereas CA 1.36 μmol/100 g. Both VA and CGA amounted a value less than 1 μmol/100 g (Table 3). With the exception of *p*-HBA, whose amount was comparable in both wheat products, the bran showed a higher contents of VA, SA, GA, PCA and FA in comparison with white flour. In particular, VA and SA were two-fold higher whereas GA, PCA and FA were 5-fold, 9-fold and 30-fold higher in bran than in white flour (Tables 3-4).

Most of phenolic acids were not affected by organic cultivation in comparison with conventional system (Tables 3-4). However, in the bran from OS the CGA content increased by about 3-fold and in white flour from OS the *p*-CA reached detectable levels, contrary to what happened for the conventional system. FA was not affected by organic cultivation in the white flour but was subjected to a decrease of 31% in the bran (Table 3).

4. Discussion

285 The question how the farming systems, including organic one, affect the crop
286 performance, as well as the nutrient value of food, is still unresolved. This fact is
287 prevalently due to the high variability in agricultural data, which results from uncontrolled
288 factors of production, first of all climate and soil. The results of the present experiment
289 were in line with those of many other studies, which reported a lower grain yield and
290 quality in wheat organically grown than conventionally one (Váňová, Klem, Míša,
291 Matušinsky, Hajšlová and Lancová, 2008).

292 Generally, the two main factors limiting yields and grain quality of organic wheat
293 are the weed competition and the nitrogen shortage. In our case, the great yield reduction
294 observed in OS may not be related to weed competition, as demonstrated by data of weed
295 biomass at wheat maturity (data not shown), which were very similar for the two systems.
296 On the contrary, the peculiar rainfall distribution recorded in the crop season could cause a
297 noticeable nitrogen shortage which penalized more the yields of OS than CS, due to the
298 different strategy of fertilization adopted for the two systems. In the conventional system,
299 high amount (155 U/ha) of soluble inorganic nitrogen was applied twice during the crop
300 growth. In the OS instead, the nitrogen, in organic form, was distributed in small quantities
301 (30U/ha) and only once, before the sowing.

302 The quality of winter wheat is determined by numerous factors, among which,
303 thousand kernel weight (TKW) and grain protein content play a major role (Ceseviciene,
304 Slepetiene, Leistrumaite, Ruzgas, & Slepetys, 2012). In our case, these factors were not
305 significantly depleted by organic cultivation (Table 1). These results are consistent with
306 what previously published by our group (Mazzoncini, Belloni, Risaliti, & Antichi, 2007)
307 and by others (Hildermann, Thommen, Dubois, Boller, Wiemken, & Mäder, 2009).

308 On the contrary, Ceseviciene et al. (2012) found a significant depletion of protein
309 content and sedimentation volume for four different bread wheat cultivars organically

310 grown. This inconsistency can be explained with a different ability of cultivars to adapt to
311 organic growing conditions.

312 Despite the fact that cv. Bologna is one of the wheat genotypes most used in Italy
313 for bread production, in our experiment its white flour resulted to have a poor bread-
314 making quality (Table 2). In particular, the flour from organic wheat contained
315 significantly less proteins than the conventional one (81.0 and 101.7 g proteins kg^{-1} d.m.
316 for OS and CS, respectively). Thus, this flour resulted more suitable for the preparation of
317 products requiring dough with low viscoelasticity such as biscuits, crackers and brittle
318 sweets.

319 The poorer bread-making quality of flour from organic wheat might have been due
320 not only to the lower content in proteins, but also to their different composition, in
321 particular to the amount of gliadin and glutenin which are the most important components
322 of gluten.

323 From a nutraceutical point of view, organic cultivation did not affect the contents of
324 total phenols and total phenolic acids both in the white flour and bran (Fig. 1). In the white
325 flour, the antioxidant power determined both with DPPH and $ABTS^{+}$ radicals, was not
326 significantly affected by the cropping system, whilst in the bran it showed higher values
327 under OS than CS (Fig. 2). In both systems, the ca. double antioxidant activity measured in
328 the bran was in agreement with the 4-7 fold higher content of phenols and phenolic acids
329 compared to the white flour (Figs. 1-2), confirming the higher nutritional quality of the
330 former.

331 According to what reported by Picchi, Migliori, Lo Scalzo, Campanelli, Ferrari,
332 and Di Cesare et al. (2012), the content in phenolic compounds is influenced by several
333 factors (e.g. exposure to pathogens, nutrient shortage, adverse weather conditions, etc.), all
334 linkable with the nutrients availability. The lower level of nitrogen fertilization under OS
335 might have been the factor responsible for the increase of the phenols content in the

organic products. In fact, the nutrients availability may affect the secondary metabolites production.

In our study the concentrations of total phenols found in the white flour and the bran (Fig. 1, Table 3) were similar to those reported by Okarter, Liu, Sorrells and Liu (2010) for different wheat varieties, ranging from 300 to 1500 μmol GAE/100 g. The OS adoption changed the composition in phenolic acids: in the white flour, the percentage of SA increased by 20% (Table 3), whereas in the bran, the percentage of FA (the main representative acid in this wheat product) was reduced from 60 to 49%.

The huge variability in phenols and in the composition in phenolic acids from that reported in the literature depends by the tested varieties (Menga et al., 2010) and the different methods (solvents, times and duration of extraction, antioxidants present during the extraction) used for the extraction (Nardini, Cirillo, Natella, Mencarelli, Comisso, & Scaccini, 2002).

The agronomic management may also explain the variability in phenolic contents. In literature the lack of plant nutrients is mentioned as a possible cause of alteration in phenolic content and composition for several crops, although most of the studies conclude that its effect is difficult to prove (Gasztonyi et al., 2011; Mpofu et al., 2006; Rembiałkowska, 2007). In fact, only the cultivation in strictly controlled conditions (as in growth chambers) can allow us to exclude the influence of different environmental factors (e.g. UV radiation) on secondary metabolites. Besides the effects of other potential co-variables (e.g. weather conditions, pest incidence), the effect of nutrient shortage on phenolic content might be also due to the complexity of plant response to nutrient availability. According to the so called "*growth/differentiation balance*" theory, plants always optimise the available resources devoting them to growth or differentiation processes (primary or secondary metabolism) also in relation to the peculiar needs expressed in each crop stage (Rembiałkowska, 2007). Thus, the nitrogen shortage limiting

362 the plant growth can promote the biosynthesis of phenols as much as these are required by
363 the physiology of the plant (Rembiałkowska, 2007).

364 The same extraction method applied to cv. Bologna showed a different composition
365 in phenolic acids between white flour and bran (Tables 3 and 4), resulting necessarily in a
366 different antioxidant power (Fig. 2). In our case, the bran showed a higher phenol content
367 (Fig. 1, Table 3); as matter of fact the majority of phenols in wheat grain is concentrated in
368 the cell wall associated to the polysaccharides and lignans materials, which are lost during
369 milling and flour refining (Adom et al., 2005; Mattila et al., 2005; Naczk & Shahidi, 2006;
370 Vaher et al., 2010). Since a close correlation between the phenolic composition and the
371 antioxidant power has been demonstrated (Sgherri et al., 2010) we can draw the conclusion
372 that a different antioxidant potential and, thus, different healthy properties exist between
373 flour and bran. This finding stresses also the importance of the presence of wheat bran in
374 food products and then in human diet, due to its high nutritional quality in addition to other
375 well-known beneficial effects on human health (low cholesterol, maintenance of intestine
376 and heart functionality, prevention of osteoporosis, above all).

377 Bran showed also the peculiar presence of CGA and CA, which further contribute
378 to increase the nutraceutical value of this milling product compared to white flour. In
379 particular, the presence of CGA, due to its polyhydroxy nature, contributes significantly to
380 the antioxidant activit; in fact, the hydroxyl derivatives of cinnamic acid appear to be more
381 powerful antioxidants than the hydroxyl derivatives of benzoic acid. In addition, they are
382 also efficient radical scavengers *in vitro* because of their capability to inhibit lipid
383 peroxidation due to their H-donating abilities as well as their partition coefficients
384 (Sgherri, Kadlecová, Pardossi, Navari-Izzo, & Izzo, 2008). The importance of CGA in
385 counteracting the damaging effects of Reactive Oxygen Species (ROS) is confirmed by its
386 unusually elevated contents in plants exposed to physical injuries, infections or other
387 stresses (Sgherri et al., 2008). As a consequence, some stressful conditions, depending on

388 the type and intensity could be seen as a way to increase the nutritional value of a plant
389 product (Sgherri & Navari-Izzo, 1995).
390 In agreement with the results obtained previously by our group and by other
391 authors, both for wheat and other crops, we did not find significant differences between the
392 two cultivation systems in terms of phenolic concentration (Kim, Tsao, Yang, & Cui,
393 2006; Mazzoncini et al., 2007; Nguyen & Niemeyer, 2008; Stracke et al., 2009; Zuchowski
394 et al., 2009, 2011). The effect of cropping system (C) as well as that of the interaction
395 between product and cropping system (P x C) were significant only for the ferulic acid
396 (FA) and the chlorogenic acid (CGA).
397 Consistently with us, Stracke et al. (2009) found no differences in the content of
398 several antioxidants and, particularly, in phenolics between organically and conventionally
399 grown wheat. The same conclusions were also achieved in 2009 by Zuchowski et al., who
400 compared the phenolic composition of several wheat genotypes grown under organic and
401 conventional conditions. On the contrary, in a more recent paper from the same team
402 (Zuchowski et al., 2011), organic wheat varieties showed higher phenolic content than the
403 conventional ones. In our conditions and during the experimental year, wheat faced very
404 few environmental stresses, except for the above mentioned nitrogen shortage. The
405 absence of unfavourable events reduced the differences between the two systems. Thus,
406 the biosynthesis of phenols might have been depressed or, alternatively, expressed poorly
407 in seeds rather than in other plant tissues, as hypothesized also by Stracke et al. (2009).
408 This makes necessary to have results from long-term experiments carried out in as
409 many as possible different sites. In any cases it is important to have in the same
410 experimental site the presence of crops under the different agronomic managements so as
411 to make possible relative comparisons among them.
412
413 **Conclusion**

The phenolic content of organic wheat were not different from those of the conventional one. Nevertheless, crop yield and baking quality were higher for conventional wheat, likely due to a greater availability of nitrogen. The nitrogen shortage, however, could be the reason for the different composition of phenolic acids and the higher antioxidant power found in the bran from organic system. Stress conditions may affect the composition in secondary metabolites of plants, and their removal may not be desirable from the point of view of the nutraceutical value of food. For some phenolic acids, such as CGA, which is an important antioxidant, we demonstrated a significant interaction between the cultivation systems and the wheat products (C x P). This confirms: i) the importance of evaluating simultaneously the effects due to the two experimental factors; ii) the need of new research on the differentiation of milling processes, aiming to the overexpression of desired phenolic compounds.

In general terms, the bran confirmed to be the wheat product most rich in phenolics, thus most healthful, irrespective of the adopted cultivation system. Its presence should be useful to increase the nutraceutical value of wheat products. This is an important result in the light of the fact that bakery processes are reputed to not alter the phenolic composition of raw material. The entire chain of food production should therefore be considered in order to produce wheat products of ever-increasing quality.

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544 **Fig. 1.** Total phenols (as determined by Folin Ciocalteu assay) and total phenolic acid
545 contents (as determined by HPLC-UV, UV) of alkaline hydrolyzed white flour (WF) and
546 bran (B) from winter wheat (cv. Bologna) grown under organic (OS) or conventional (CS)
547 system. Columns and bars represent mean ± standard deviation (n = 3). A two-way
548 ANOVA was used to evaluate the effect of the wheat product (P), the effect of the
549 cultivation system (C) and the interaction between wheat product and cultivation system (P
550 x C); p was always < 0.05 (LSD test). Significance was as follows: ns, not significant; *,
551 significant at the $p \leq 0.05$ level; **, significant at the $p \leq 0.01$ level. GAE, gallic acid
552 equivalents.
553
554 **Fig. 2.** Antioxidant properties of white flour (WF) and bran (B) from winter wheat (cv.
555 Bologna) grown under organic (OS) or conventional (CS) system. DPPH (1,1-diphenyl-2-
556 picrylhydrazyl) radical scavenging and $ABTS^{+}$ (2,2'-azinobis[3-ethylbenzothiazoline-6-
557 sulfonic acid]) radical cation scavenging were expressed as mmol trolox equivalents (TE)
558 per 100 g of product. Columns and bars represent mean ± standard deviation (n = 3). A
559 two-way ANOVA was used to evaluate the effect of the wheat product (P), the effect of
560 the cultivation system (C) and the interaction between wheat product and cultivation
561 system (P x C). Significance was as follows: *, significant at the $p \leq 0.05$ level; **,
562 significant at the $p \leq 0.01$ level.
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564

Table 1

Table 1
Biomass parameters (number of ears, dry matter production, thousand kernel weight, hardness, total protein content and SDS sedimentation volume) of grains from winter wheat (cv. Bologna) grown under organic (OS) or conventional (CS) system. TKW, thousand kernel weight; SDS, sodium dodecyl sulphate; d.m., dry matter.[a]

Systems	Number of ears (m^{-2})	Dry matter yield (Mg d.m. ha^{-1})				TKW (g)	Hardness	Protein ($g\ kg^{-1}$)	SDS (mL)
		Straw	Ears	Grain	Total				
CS	551	4.06	1.26	4.85	10.17	27.55	75.33	111.70	43
OS	520	2.40	0.77	2.65	5.82	26.69	61.67	98.30	33
Significance	ns	*	*	*	*	ns	ns	ns	ns
LSD	201	1.32	0.49	1.52	3.30	2.79	39.85	46.00	38

[a] Significance was as follows: ns, not significant; *, significant at the $p \leq 0.05$ level (LSD test).

Table 2
Characteristics of white flour obtained from winter wheat (cv. Bologna) grown under organic (OS) or conventional (CS) system. W and P/L, alveographic indexes.[a]

Systems	Protein content ($g\ kg^{-1}$)	Water content ($g\ kg^{-1}$)	W (10^{-4} J)	P/L	Falling number (s)
CS	101.7	145.3	212.00	0.89	339.33
OS	81.0	147.8	104.33	1.35	335.33
Significance	*	ns	*	ns	ns
LSD	12.3	17.3	68.68	0.92	15.27

[a] Significance was as follows: ns, not significant; *, significant at the $p \leq 0.05$ level (LSD test).

Table 3

Table 3
Phenolic acid content (μmol/100 g) in white flour and bran obtained from wheat (cv. Bologna) grown under organic (OS) or conventional (CS) system. All data are reported as mean ± standard deviation (n = 3).[a]

Wheat products	Cropping systems	GA	PCA	*p*-HBA	VA	SA	*p*-CA	FA	CGA	CA
White flour	CS	1.24 ± 0.81	4.20 ± 2.79	1.62 ± 0.64	0.41 ± 0.30	21.83 ± 8.73	nd	4.95 ± 3.50	nd	nd
White flour	OS	0.21 ± 0.12	4.83 ± 3.77	0.76 ± 0.25	0.46 ± 0.29	30.74 ± 8.92	0.58 ± 0.43	1.72 ± 1.32	nd	nd
Bran	CS	5.92 ± 2.28	36.10 ± 5.69	1.51 ± 0.70	0.93 ± 0.27	47.60 ± 3.66	3.52 ± 1.22	149.96 ± 8.59	0.84 ± 0.45	1.36 ± 0.63
Bran	OS	7.04 ± 2.17	37.53 ± 4.56	1.84 ± 0.45	0.53 ± 0.31	54.92 ± 2.29	3.12 ± 0.78	103.47 ± 23.73	2.72 ± 0.89	1.51 ± 0.91

[a] GA, gallic acid; PCA, protocatechuic acid; *p*-HBA, *p*-hydroxybenzoic acid; VA, vanillic acid; SA, syringic acid; *p*-CA, *p*-coumaric acid; FA, ferulic acid; CGA, chlorogenic acid; CA, caffeic acid; nd, not detected.

Table 4
Effect of wheat product (P), cultivation system (C), and the interaction between P and C (P x C) on phenolic acid content (μmol/100 g) in white flour and bran obtained from wheat (cv. Bologna) grown under organic (OS) or conventional (CS) system.[a]

	P	C	P x C
GA	89.02 *	0.01 ns	3.09 ns
PCA	97.67 *	0.10 ns	0.02 ns
p-HBA	26.60 ns	7.90 ns	40.09 ns
VA	33.97 *	11.95 ns	19.14 ns
SA	85.23 *	9.00 ns	0.09 ns
p-CA	90.98 *	0.06 ns	2.29 ns
FA	92.41 **	3.75 *	2.84 *
CGA	60.96 *	17.12 *	17.12 *
CA	83.35 ns	0.23 ns	0.23 ns

[a] The results were expressed as percent of total mean square. Significance was as follows: ns, not significant; *, significant at the $p \leq 0.05$ level; **, significant at the $p \leq 0.01$ level. GA, gallic acid; PCA, protocatechuic acid; *p*-HBA, *p*-hydroxybenzoic acid;VA, vanillic acid; SA, syringic acid; *p*-CA, *p*-coumaric acid; FA, ferulic acid; CGA, chlorogenic acid; CA, caffeic acid.

Figure 1
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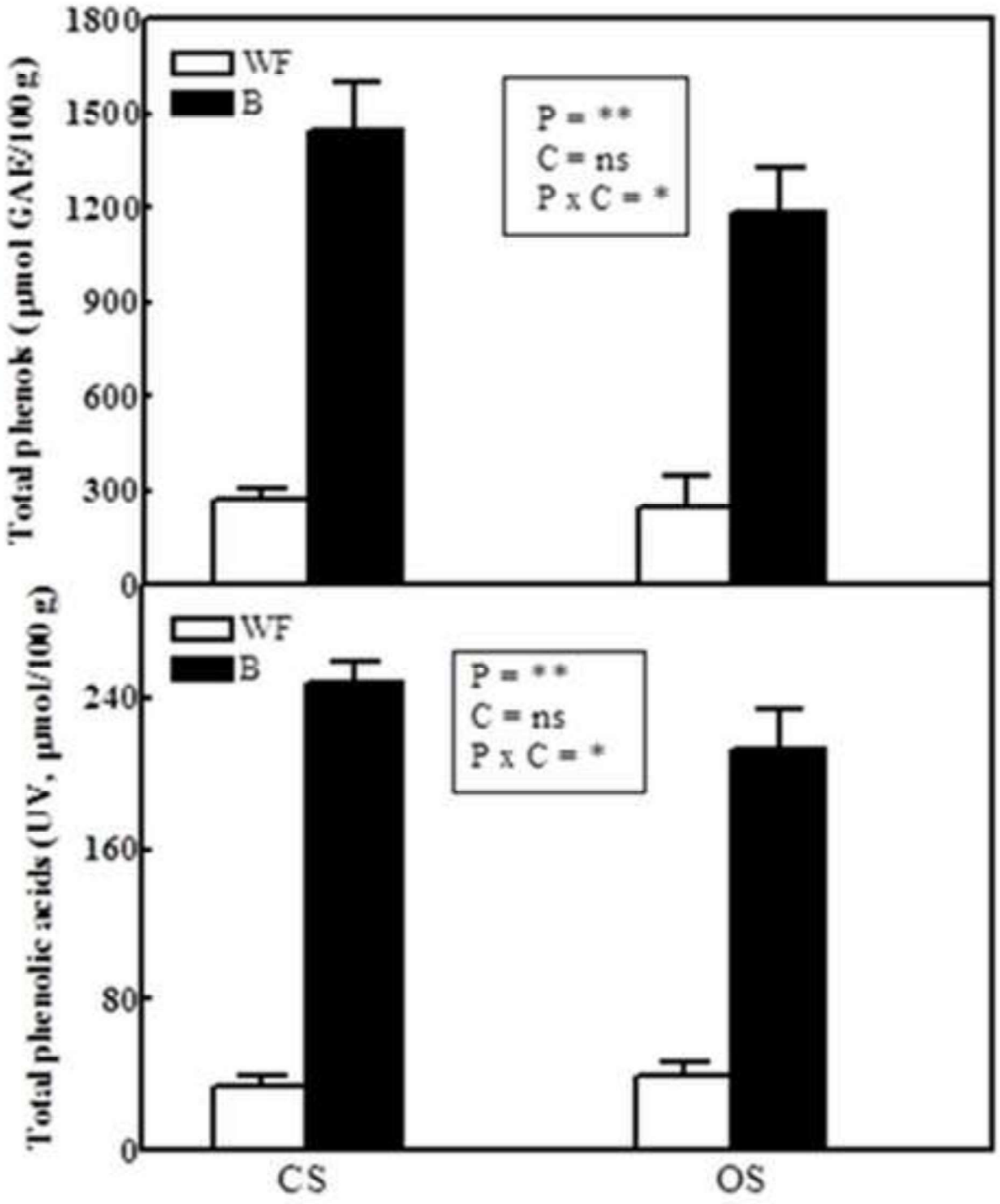

Figure 2

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