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**Folate distribution in barley (*Hordeum vulgare* L.), common wheat (*Triticum aestivum* L.) and durum wheat (*Triticum turgidum durum* Desf.) pearled fractions**

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(Article begins on next page)



## UNIVERSITÀ DEGLI STUDI DI TORINO

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33 **Title: Folate distribution in barley (*Hordeum vulgare* L.),**  
34 **common wheat (*Triticum aestivum* L.) and durum wheat**  
35 **(*Triticum turgidum durum* Desf.) pearled fractions**

36

37 **Running title: Folate distribution in barley and wheat pearling fractions**

38

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## ABSTRACT

**Background:** Wholegrain cereals are an important source of folates. In this study, total folate was analyzed in different pearling fractions of hulled and hulless barley as well as in common and durum wheat in order to evaluate its distribution in the kernels.

**Results:** A noticeable variation in the folate content was observed between the barley and wheat varieties. The highest folate content was detected in the hulless barley variety. A significant reduction in total folate, from 63% to 86%, was observed in both barley and wheat varieties from the outermost to the innermost pearling fractions.

**Conclusion:** Results have proved that folates are mainly present in the germ and in the outer layers of the kernel. This is the first study reporting the folate distribution in both common and durum wheat and in hulless barley varieties. Results suggest that the folate content could be naturally enhanced by introducing grain pearling fractions into cereal food products.

## KEYWORDS

Folates, Vitamin B9, Common wheat, Durum wheat, Barley

## INTRODUCTION

84

85 Folates, also known as vitamin B9, are a group of water-soluble vitamins  
86 characterized by a similar biological activity to folic acid. Folates are naturally  
87 present in some food, are added to others, and are available as dietary  
88 supplements. This family of vitamins is currently one of the most actively  
89 studied, because of its pivotal role as an essential coenzyme to provide one-  
90 carbon units for nucleotide biosynthesis, amino acid metabolism and DNA  
91 methylation in organisms.<sup>1</sup> Unlike plants and microorganisms, animals cannot  
92 synthesize folates *ex novo* and depend entirely on their dietary supply. Although  
93 folates are omnipresent in a normal human diet, folate intake often falls below  
94 the recommended levels, even in highly-developed countries.<sup>2,3</sup> An insufficient  
95 folate intake is associated with a large number of health disorders, such as  
96 megaloblastic anemia,<sup>4</sup> and with an increased risk of Neural Tube Defects  
97 (NTDs) in the developing foetus.<sup>5</sup> In addition, there is scientific evidence of a  
98 relationship between folate deficiency and several other diseases, such as  
99 cardiovascular diseases,<sup>6</sup> Alzheimer's disease<sup>7</sup> and some forms of cancer.<sup>8</sup>  
100 The gap between the recommended and actual folate intake has led to  
101 mandatory folic acid food fortification in more than 60 countries around the  
102 world, but not in Europe, where only voluntary food fortification is practiced [Reg.  
103 (EC) No 1925/2006]. Although folic acid food fortification has been the main  
104 strategy undertaken over the years to increase folate diet levels, natural folate  
105 enhancement has recently also attracted attention in countries where  
106 mandatory folic acid food fortification is not practiced. Variations in the folate  
107 content of wheat,<sup>9</sup> barley,<sup>10</sup> oat<sup>11</sup> and rye<sup>12</sup> were examined in the  
108 comprehensive European HEALTHGRAIN project. Information is also currently  
109 available on folate levels in rice<sup>13</sup> and pseudocereals.<sup>14</sup> Even though cereal

110 grains and their derived products are important sources of natural folates, the  
111 folate content of cereal products depends on both the initial grain content and  
112 the severity of the grain milling process. In fact, bioactive compounds, such as  
113 folates, are unevenly distributed in cereal grains. As reported in previous  
114 studies, folates are mainly concentrated in the bran and in germ fractions.<sup>15-17</sup>  
115 These fractions are generally removed during the milling process and remain in  
116 the bran fraction, which is mainly used for feeding. Hegedüs and collaborators<sup>18</sup>  
117 showed that, with an extraction rate of 87% in the milling process, the wheat  
118 flour folate concentration is reduced to 79% of that of wholegrain flour. Similarly,  
119 with an extraction rate of 66%, the wheat flour folate concentration is reduced to  
120 10% of that of wholegrain flour. These data are in agreement with other results  
121<sup>19</sup> which show that the folate content is reduced remarkably in sifted wheat  
122 flours with extraction rates of 70-80%. Similarly, Arcot and collaborators<sup>15</sup>  
123 reported that wheat bran folate levels are 4-fold higher than those of flour and 2-  
124 fold higher than those of grain.

125 For these reasons, the “whole grain concept” promotes the consumption of all  
126 the grain components in the same proportion as in the native grain.<sup>20</sup> However,  
127 the outer layers of the grain may confer undesirable properties to bakery  
128 products, in terms of safety, processing, or acceptability by consumers. The  
129 outer layers of the wheat kernel are the most prone to contamination by  
130 mycotoxins, heavy metals and pesticides.<sup>21</sup> Moreover, wholegrain foods are not  
131 so attractive to consumers, because of the high bran content in wholegrain flour,  
132 which reduces the sensory value of the end-use products.<sup>22</sup> Therefore, the  
133 cereal grain fractionation technology, which could also be applied easily with a  
134 selective dry pearling process, is receiving a great deal of attention because of  
135 its capacity to efficiently separate the negative and positive aspects, in order to

136 produce new ingredients and flour mixes with technologically optimized  
137 functional and nutritional attributes.<sup>23</sup> This will allow to produce new flour mixes  
138 and ingredients with technologically optimized functional and nutritional  
139 attributes. The pearling (debranning) of wheat, before roller milling, is going to  
140 be increasingly accepted by wheat millers to improve milling performance, since  
141 it sequentially removes the outer kernel bran layers through an abrasive  
142 scouring and increases the efficiency of the milling process.<sup>24</sup> The average  
143 concentrations of mycotoxins and heavy metals are more effectively reduced by  
144 pearling than milling.<sup>21</sup> Moreover, the degree of pearling could be carefully  
145 modulated to separate the external bran fractions, which are characterized by a  
146 higher toxicity risk and coarse fiber, from the cereal fractions which offer  
147 potentially high health benefits. In this way, it would therefore be possible to  
148 enrich conventional flour with cereal bran fractions, obtained from sequential  
149 pearling and characterized by higher antioxidant activity and bioactives content,  
150 but also by a lower risk of contamination.<sup>25</sup> For this reason, the distribution of  
151 the different nutrients in the cereal grain should be evaluated.

152 Although the distribution of folates in hulled barley fractions was already  
153 investigated,<sup>26</sup> to the best of the authors' knowledge no information is available,  
154 in the scientific literature, on the distribution folates in pearling fractions of either  
155 common or durum wheat or hullless barley. The aim of this study was to  
156 determine the folate distribution in the kernels of two wheat and three barley  
157 cultivars, in order to enhance the achievement of grain fractions rich in these  
158 compounds through the pearling process.

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## MATERIALS AND METHODS

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### 163 **Plant material**

164 Three commercial barley varieties (*Hordeum vulgare* L.) and two wheat  
165 varieties, namely, one common wheat (*Triticum aestivum* L.), and one durum  
166 wheat (*Triticum turgidum durum* Desf.), were cultivated side by side on the  
167 same field, in a medium-texture fertile soil in Carignano, Piedmont, NW Italy  
168 (44°53'8.69"N, 7°41'16.75"E, 232 m a.s.l.), during the 2011-12 growing season,  
169 according to the ordinary crop management program applied to these crops in  
170 the growing area.

171 The compared barley varieties were:

- 172 • Mona (S.I.S, San Lazzaro di Savena, BO, Italy): spring, hullless and two-row  
173 variety, used for food purposes;
- 174 • Trasimeno (Geo Seed, Grinzano di Cervere, CN, Italy): winter, hulled and  
175 two-row variety, used both for food and feed purposes;
- 176 • Ketos (Limagrain Italia Spa, Busseto, PR, Italy): winter, hulled and six-row  
177 variety, mainly used for feed purposes.

178 The compared wheat varieties were:

- 179 • Generale (Consorzio Nazionale Sementi, Conselice, RA, Italy): common  
180 wheat variety classified, according to the Italian bread-making quality grade,  
181 <sup>27</sup> as superior bread making-quality wheat;
- 182 • Colombo (Apsovsementi S.p.A., Voghera, PV, Italy): durum wheat variety  
183 classified as high quality wheat.

184 Planting was conducted in 12 cm wide rows at a seeding rate of 450 seeds m<sup>-2</sup>  
185 on October 24, 2011 for the winter barley and wheat, while cv. Mona was  
186 planted on March 1, 2012. The previous crop was maize for grain. Fungicide  
187 treatments were only performed on the common and durum wheat to avoid the



188 development of foliar and head fungal diseases at stem elongation (GS-35, a.i.  
189 azoxystrobin and cyproconazole applied at 0.2 kg ha<sup>-1</sup> and 0.08 kg ha<sup>-1</sup>,  
190 respectively) and at heading (GS-58, a.i. prothioconazole applied at 0.250 kg  
191 ha<sup>-1</sup>).

192 One hundred and thirty and 170 kg N ha<sup>-1</sup> were applied as granular ammonium  
193 nitrate fertilizers for the barley and wheat cultivars, respectively. Harvesting was  
194 conducted with a combine-harvester on June 23 and July 10, 2012 for the  
195 barley and wheat cultivars, respectively. Kernel samples of each cultivar were  
196 stored at 4°C until the beginning of the tests.

197

### 198 **Barley and wheat grain pearling**

199 From 6 to 9 pearling fractions of the kernels were obtained through the  
200 incremental pearling of the barley and wheat varieties, according to the  
201 approach described by Beta and collaborators.<sup>28</sup> The pearling consisted of  
202 consecutive passages of kernels and pearled kernels in an abrasive-type grain  
203 testing mill (Model TM-05C, Satake, Tokyo, Japan) at a constant speed of 55  
204 Hz. The pearling process was monitored by means of a time control. After each  
205 assay, the laboratory pearler was cleaned thoroughly to minimize equipment  
206 contamination. Initially, a 500-g portion of each unprocessed sample was sub-  
207 sampled from a 5-kg sample, and the remaining 4.5 kg was pearled.

208 A different number of bran fractions was obtained for the barley, according to its  
209 hulled or hullless nature, in order to obtain a similar kernel pearling degree.  
210 Starting from unprocessed hullless barley grain (cv. Mona), the kernels were  
211 initially pearled to remove 5% of the original grain weight, and this resulted in a  
212 first fraction (0-5%). Then, the remaining kernels were pearled to remove a  
213 second fraction of 5% (5-10%). The pearling process was repeated to remove a

214 third, fourth and fifth fraction (designed fractions of 10-15%, 15-20%, 20-25%).  
215 A residual 75% of the kernel (25-100%) was also collected.  
216 Kernel fractions of 5% in weight were obtained in the hulled barley varieties.  
217 Nevertheless, in this case, the first two passages mainly removed the hull  
218 fractions. The corresponding fractions of the hulless barley were obtained from  
219 the third pearling passage. Thus, these pearled fractions were called hull1, hull2,  
220 0-5%, 5-10%, 10-15%, 15-20%, 20-25%, 25-30% and 30-100%.  
221 The pearling process for the wheat varieties was the same described for the  
222 hulless barley variety. The kernels were pearled in order to obtain 6 pearling  
223 fractions (see above).  
224 The whole unprocessed grain samples and the residual fractions were milled  
225 using a laboratory centrifugal mill (Model ZM-100, Retsch, Haan, Germany) with  
226 a 1-mm opening. All the samples were stored at -25°C before the chemical  
227 analyses were performed.

228

### 229 **Chemical analyses**

230 The moisture and the total folate content were determined on ground whole  
231 kernels and their pearled fractions. The moisture content was obtained, using a  
232 Sartorius MA30 thermo-balance (Sartorius AG, Goettingen, Germany), in order  
233 to express the results on a dry matter (dm) basis.

234 The total folate content was determined using a microbiological assay,  
235 according to the AOAC Official Method 2004.05, with a few modifications. This  
236 method is applied to cereal grains and cereal grain foods containing added  
237 folates (folic acid) or natural occurring folates with levels of 7.6  $\mu\text{g } 100 \text{ g}^{-1}$  to  
238 100% folates.

239 Briefly, a 1.0 g sample was mixed thoroughly with a phosphate buffer, pH 7.8  
240 (0.1 M sodium phosphate dibasic, 1% ascorbic acid) and then diluted with water  
241 to a 50 ml volume. Samples were autoclaved for 15 min at 121-123°C and  
242 treated with Termamyl®Ultra 300 L (Novozymes, Denmark). The homogenate  
243 was then treated with human plasma conjugase (Sigma-aldrich, Saint Luis,  
244 Missouri) and Creon 10000 [8000 EP-e amylase, 10000 EP-e lipase and 600  
245 EP-e protease (Abbott Healthcare Products, Maidenhead, United Kingdom)].  
246 The samples were treated for 3 min at 100°C and then cooled. The pH was  
247 adjusted to 4.5 and the samples were then diluted to a volume of 100 ml and  
248 filtered through a 2V Whatman filter paper.

249 The total folate content was determined through a microbiological assay with  
250 *Lactobacillus casei* subspecies *rhamnosus* (ATCC 7469). A folic acid-free  
251 double strength basal medium was added to each tube and then autoclaved for  
252 6 min at 121-123°C. The tubes were rapidly cooled to minimize browning  
253 reactions between the amino acids and sugars, and then aseptically inoculated  
254 with a 50 µl drop working inoculum. After incubation at 37°C for 22 h, bacteria  
255 growth was measured as optical density at 595 nm using inoculated blanks as  
256 the reference blank. The amount of folates in each sample was determined  
257 through a comparison with calibration solutions of known concentrations.

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### 259 **Statistical analyses**

260 All the analyses were performed in triplicate. One-way analysis of variance  
261 (ANOVA) was applied separately for each cultivar in order to compare the folate  
262 content among the different wheat and barley varieties and among the pearling  
263 fractions. The residual normal distribution was verified using the Shapiro-Wilk  
264 test, while variance homogeneity was verified by performing the Levene test.

265 The REGW-Q test was performed for multiple comparison purposes. When the  
266 ANOVA assumptions were not verified, rank transformation of the data was  
267 performed.<sup>29</sup> SPSS for Windows statistical package, Version 20.0 (SPSS Inc.,  
268 Chicago, Illinois) was used for the statistical analyses. A 0.05 threshold was  
269 used as the cut-off value for significance in all the tests.

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## RESULTS and DISCUSSION

291

### 292 **Total folate content in barley and wheat cultivars**

293 The comparison of folate content of the different barley and wheat varieties  
294 showed significant differences (Table 1).

295 Three barley varieties were selected for the study, considering spring and winter  
296 types, as well as two-row and six-row types. The total folate content of the three  
297 genotypes was, on average, 806 ng g<sup>-1</sup> dm, and ranged between 653 and 1033  
298 ng g<sup>-1</sup> dm. The mean folate level observed in this study fell within the range  
299 reported in previous studies for this crop (518-918 ng g<sup>-1</sup> dm<sup>10,26</sup>). The lowest  
300 folate content was observed in the hulled winter barley cv. Ketos (653 ng g<sup>-1</sup>  
301 dm), which is normally used for animal feeds. A similar folate level was  
302 observed in cv. Trasimeno (732 ng g<sup>-1</sup> dm), which is also intended for the food  
303 chain. Conversely, a significantly higher folate content was observed in the  
304 hulless barley cv. Mona (1033 ng g<sup>-1</sup> dm). Andersson and collaborators,<sup>10</sup>  
305 comparing 10 barley varieties, including 2 hulless and 8 hulled, observed that  
306 there is no clear trends in the folate content, when either winter or spring  
307 varieties or hulled and hulless ones are considered. Thus, other genetic  
308 components would seem to play a more important role. Recently, hulless barley  
309 has gained particular attention for food purposes because of the content of  
310 several other nutritional compounds, in particular β-glucans, which is higher  
311 than in hulled barley varieties.<sup>30</sup> Thus, the identification of hulless varieties rich  
312 in both folates and β-glucans could increase the nutritional value of barley food  
313 products.

314 The total folate content in the common and durum wheat cultivars was, on  
315 average, 1072 ng g<sup>-1</sup> dm. Both cultivars were characterized by a similar folate  
316 content (1024 ng g<sup>-1</sup> dm and 1119 ng g<sup>-1</sup> dm, respectively). The total folate

317 content of both common and durum wheat cultivars fell within the wide range  
318 reported in previous studies.<sup>15</sup> However, the results were over the range  
319 reported by Piironen and collaborators<sup>9</sup> for 130 winter wheat cultivars (mean  
320 561 ng g<sup>-1</sup> dm, range 364-774 ng g<sup>-1</sup> dm) and for 10 durum wheat cultivars  
321 (mean 741 ng g<sup>-1</sup> dm, range 637-891 ng g<sup>-1</sup> dm) cultivated in Hungary. The  
322 folate level in the wheat grains was shown to vary over a wide range. The  
323 variation in folate content that we observed could partly be explained by the  
324 different genetic backgrounds or growing conditions.<sup>9</sup> The folate content of  
325 another common wheat cultivar grown in climatic and agronomic conditions  
326 similar to the ones described above was shown to be lower (659 ng g<sup>-1</sup> – data  
327 not showed) than those observed for cvs. Generale and Colombo.

328

### 329 **Total folate content in barley pearling fractions**

330 The folate content in the fractions obtained from the sequential barley pearling  
331 is reported in Figure 1. Folate-rich fractions were achieved for both the hulled  
332 and hullless cultivars by pearling.

333 The Trasimeno and Ketos hulled cultivars were characterized by covered  
334 kernels, and the hulls were mainly removed in the first two pearling passages.  
335 Thus, the first two fractions (hull1 and hull2) mainly consisted of lignocellulosic  
336 material characterized by a high mineral content.<sup>31</sup> The folate concentration in  
337 the first two pearling fractions for both cv. Ketos and cv. Trasimeno was not  
338 significantly different from that of the whole grain. The authors hypothesized  
339 that total folates in the first two fractions were mainly derived from the germ and  
340 pericarp tissues. Since the germ is located at the proximal end of the barley  
341 kernel, as reported by Edelmann and collaborators,<sup>26</sup> tiny fragments were  
342 apparently removed within the first two fractions, as demonstrated by the

343 presence of white germ particles in the fibrous hull. Furthermore, in the second  
344 pearling fraction, a part of the folates might have come from the seed coats  
345 (pericarp and testa). Thus, all these facts could be responsible for the high total  
346 folate content observed in the hull fractions. After hull removal, a characteristic  
347 folate distribution was observed in both hulled barley cultivars and a progressive  
348 reduction was observed from the outermost to the innermost layers. In fact, in  
349 both cultivars, the highest folate concentration was observed in the 0-5%  
350 pearling fraction, which corresponds to the outermost layers of the kernel. In  
351 particular, the 0-5% fraction of cv. Trasimeno was characterized by a folate  
352 concentration of 1038 ng g<sup>-1</sup> dm (1.4-fold higher than the whole grain), while the  
353 Ketos one was characterized by 2393 ng g<sup>-1</sup> dm (about 4-fold higher than the  
354 whole grain). Moreover, a reduction in the folate level was observed after each  
355 progressive pearling passage towards the innermost layers. In particular, a  
356 significant reduction in the folate level was observed for the two-row barley cv.  
357 Trasimeno after the removal of 30% of the kernel weight. The mean folate level  
358 observed from the 0-5% to the 25-30% fraction was 903 ng g<sup>-1</sup> dm. The residual  
359 30-100% fraction, which mainly corresponds to the endosperm, was  
360 characterized by a folate concentration of 385 ng g<sup>-1</sup> dm. Even though the  
361 reduction in folate concentration was not significant, a gradual reduction in  
362 folate concentration was observed from the 15-20% fraction to the 25-30%  
363 fraction. This was probably due to an increase in endosperm content.  
364 Conversely, a gradual significant reduction was observed in the six-row cv.  
365 Ketos from the 0-5% fraction to the innermost one. In particular, as reported  
366 above, the 0-5% fraction, corresponding to the outer layers of the kernel, was  
367 the one that was characterized by the highest folate level. A not significant  
368 reduction of 25% was observed in the subsequent 5-10% fraction. After the

369 removal of the first two fractions, a higher reduction was observed, ranging from  
370 58% to 86%, in comparison to the 0-5% fraction. The folate distribution  
371 observed in the hulled barley varieties analyzed in this study has confirmed data  
372 reported for other hulled two-row and six-row varieties.<sup>26</sup>

373 To the best of the authors' knowledge no other work has described the folate  
374 distribution in hulless barley kernels. As shown for the hulled barley cultivars, a  
375 significant reduction in the folate content was also observed in the hulless two-  
376 row cv. Mona from the outermost to the innermost kernel layers. In particular,  
377 the highest folate concentration was found in the outermost 0-5% fraction,  
378 which is characterized by a concentration of 4647 ng g<sup>-1</sup> dm. In this fraction, the  
379 folate content was about 4.5-fold higher than in the whole grain. A high folate  
380 level (3970 ng g<sup>-1</sup> dm) was also observed in the second 5-10% fraction. This  
381 content was about 4-fold higher than that of the whole grain. The remaining  
382 parts of the pericarp and testa, but also a part of the aleurone layer and of the  
383 germ were probably collected in this fraction.<sup>26,32</sup> Thus, on the basis of previous  
384 observations on barley fractionation,<sup>26,32,33</sup> it was assumed that the high level of  
385 folate observed in the first fractions mainly came from the germ, and from the  
386 outer layers of the kernel. The folate content in the next three fractions (10-15%,  
387 15-20% and 20-25%) was still fairly high, compared to the whole grain (3350 ng  
388 g<sup>-1</sup> dm, 2652 ng g<sup>-1</sup> dm and 2009 ng g<sup>-1</sup> dm, respectively). The folates in these  
389 fractions probably mainly came from the aleurone layer, because most of the  
390 germ had been eliminated earlier. After removing 25% of the kernel weight by  
391 pearling, the germ and the outer layers of the kernel were removed, and the rest  
392 of the grain probably mainly consisted of endosperm tissues, with small  
393 amounts of aleurone and subaleurone. In fact, a significantly lower folate  
394 concentration was observed in the innermost fraction (620 ng g<sup>-1</sup> dm). The folate



395 content of the innermost residual fraction was significantly lower than that of the  
396 whole grain.

397

### 398 **Total folate content in wheat pearling fractions**

399 The folate content in the fractions obtained from the sequential wheat pearling  
400 is reported in Figure 2. As previously reported for barley, folate-rich fractions  
401 were achieved from both the common and durum wheat cultivars by pearling.

402 The folate content in the first three fractions of the common wheat was  
403 significantly higher than that of both the whole grain and of all the other pearling  
404 fractions. The folate content in these three fractions (0-5%, 5-10% and 10-15%)  
405 was 2050 ng g<sup>-1</sup> dm, 1895 ng g<sup>-1</sup> dm and 1801 ng g<sup>-1</sup> dm, respectively. The  
406 folate concentration in the first three pearling fractions was about 2-fold higher  
407 than that of the whole grain (1024 ng g<sup>-1</sup> dm). After the removal of the external  
408 fractions, a significant reduction in the folate concentration was observed; the  
409 folate concentration of the intermediate pearling fractions (15-20% and 20-25%)  
410 and of the residual fraction (25-100%) was 883 ng g<sup>-1</sup> dm, 727 ng g<sup>-1</sup> dm and  
411 726 ng g<sup>-1</sup> dm, respectively. Although no significant differences were observed  
412 between the last three fractions, a decrease in folate content was observed  
413 from the outermost fraction to the innermost one. The folate content observed in  
414 these pearling fractions was not significantly different from that of the whole  
415 grain.

416 A similar trend was also observed in the durum wheat pearling fractions. The  
417 folate content in the first three fractions (0-5%, 5-10% and 10-15%) was 2670  
418 ng g<sup>-1</sup> dm, 2651 ng g<sup>-1</sup> dm and 2765 ng g<sup>-1</sup> dm, respectively. The folate content  
419 in these three fractions was significantly higher than that of both the whole grain  
420 and of all the other fractions. This content was about 2.5-fold higher than that

421 observed in the whole grain (1119 ng g<sup>-1</sup> dm). A significant reduction in the  
422 folate level was observed after the removal of the external fractions. In fact, the  
423 intermediate pearling fractions (15-20% and 20-25%) were characterized by a  
424 folate content of 1890 ng g<sup>-1</sup> dm and 2095 ng g<sup>-1</sup> dm, respectively. However, the  
425 folate content in these two fractions was about 2-fold higher than that of the  
426 whole grain. The lowest folate content was observed in the 25-100% residual  
427 fraction (784 ng g<sup>-1</sup> dm). No significant difference was observed between the  
428 innermost fraction and the whole grain.

429 Shetlar and collaborators<sup>34</sup> reported that the outer pericarp, the inner pericarp,  
430 the testa and the aleurone layer, represent 3.9%, 0.9%, 0.7% and 9% of the  
431 kernel weight, respectively. Therefore, according to data reported in other  
432 studies,<sup>35-37</sup> pearling up to the 5% level on average removed most of the outer  
433 pericarp, while the aleurone layer was removed at the 5-10% and 10-15% level.  
434 Furthermore, as reported for barley, part of the folates observed in the first  
435 pearling fractions might have originated from the germ. The results have  
436 confirmed that the folates were mainly concentrated in the outer layers and in  
437 the germ of wheat kernels. Similarly, Hemery and collaborators<sup>17</sup> showed that  
438 the electrostatic wheat bran separation process produced a fraction that was  
439 rich in aleurone cells and which was therefore characterized by a large amount  
440 of folates (1188 ng g<sup>-1</sup> dm). Moreover, Fenech and collaborators<sup>16</sup> reported a  
441 folate concentration of between 4000 and 6000 ng g<sup>-1</sup> in wheat aleurone flour  
442 containing aleurone and germ particles, and the isolated wheat germ contained  
443 2000 ng g<sup>-1</sup> dm folates.<sup>38</sup>

444 To our knowledge, this is the first study that describes the folate distribution in  
445 pearling fractions of common wheat, durum wheat and hulless barley kernels.  
446 Even though the first pearling fractions were characterized by the highest folate

447 content in both the wheat and the hulless barley, these fractions could result in  
448 a higher sanitary risk as a consequence of the high concentrations of  
449 mycotoxins, heavy metals and pesticides.<sup>39</sup> In order to obtain a functional  
450 ingredient through wheat pearling, Sovrani and collaborators,<sup>39</sup> suggested the  
451 10-15% intermediate fraction for common wheat as the best compromise  
452 between high nutritional value and low mycotoxin contamination risks. Moreover,  
453 it was observed that the addition of this intermediate fraction with a 10%  
454 substitution level<sup>25</sup> to refined flour could increase the content of bioactives with  
455 limited effects on the technological properties. Considering our results, the  
456 addition of cvs. Colombo and Mona 10-15% fraction with a 10%-substitution  
457 level<sup>25</sup> to the refined flour could increase the folate level up to 15% of the  
458 Nutrient Reference Value [NRV – Reg. (EU) No 1169/2011]. The addition of cv.  
459 Generale 10-15% fraction under the same conditions could improve the folate  
460 level up to 9% of the NRV. Instead, the addition of barley cvs. Ketos and  
461 Trasimeno 10-15% fraction could only increase the folate level up to 5% of NRV.  
462 Further studies are necessary in order to identify the functional ingredients that  
463 would be able to enrich the folate contents in bakery products, while considering  
464 that these compounds are unstable at high temperatures and as a result their  
465 levels could decrease in the final food products.<sup>40</sup> In conclusion, our results  
466 suggest that the pearling process could be a useful and practical tool in order to  
467 select intermediate bran fractions from small cereals, as a natural source of  
468 folates, separated from detrimental components, in order to develop nutritionally  
469 enhanced ingredients and products.

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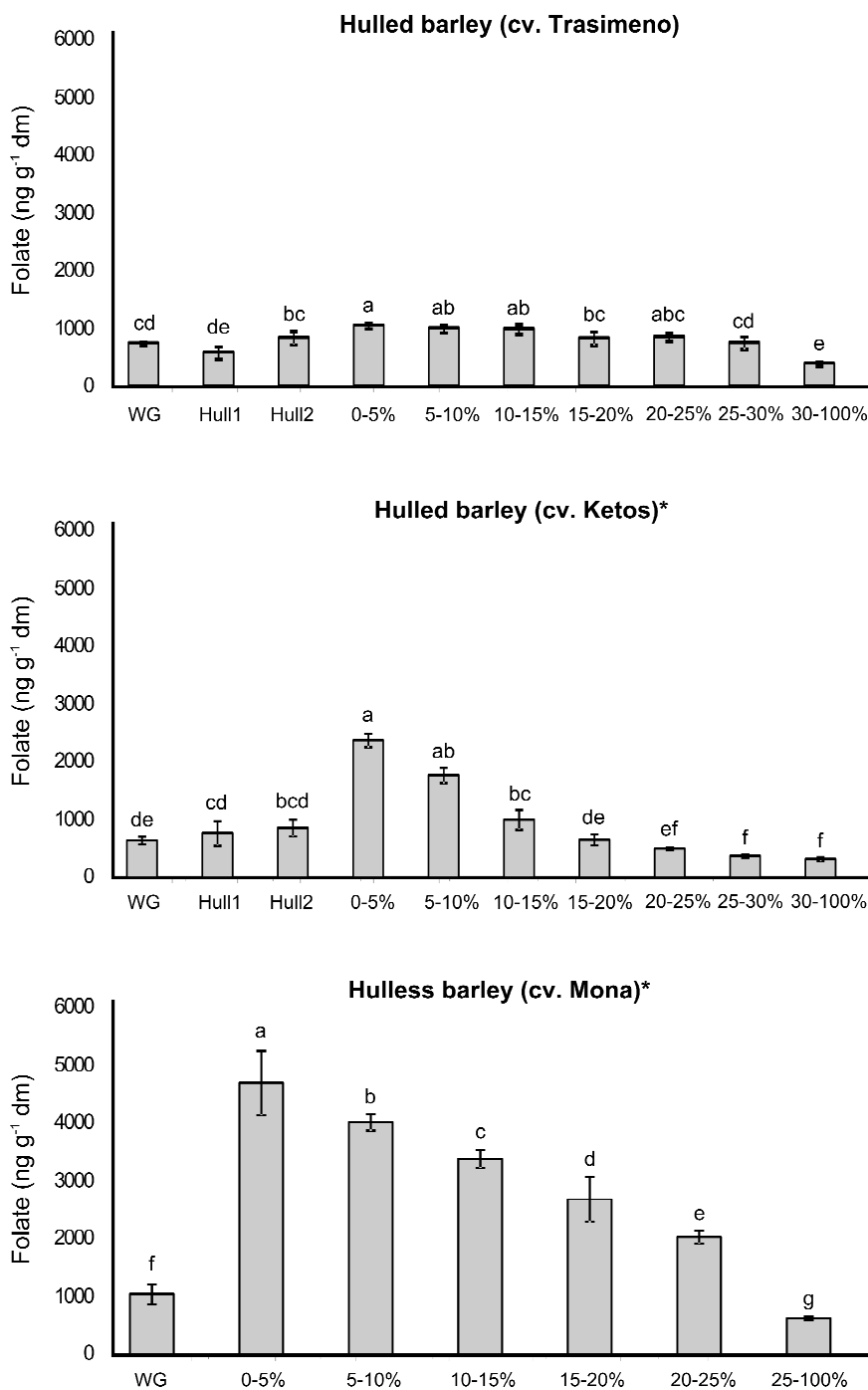
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**FIGURES**

608 **Figure 1.** Folate content in the barley pearling fractions.

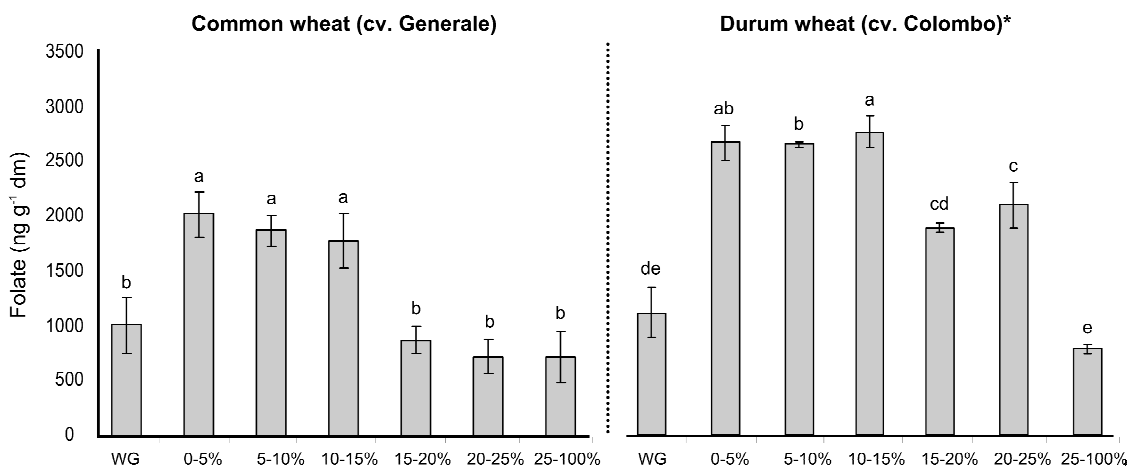


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610 The reported data are the means of three values; values with different letters  
 611 differ significantly ( $P < 0.05$ ). The error bars indicate the standard deviation. (WG:  
 612 Whole Grain, \*data analyzed after rank transformation).

613

614 **Figure 2.** Folate content in the wheat pearling fractions.



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616 The reported data are the means of three values; values with different letters  
617 differ significantly ( $P < 0.05$ ). The error bars indicate the standard deviation. (WG:  
618 Whole Grain, \*data analyzed after rank transformation).

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**TABLE**

636 **Table 1.** Folate content in the whole grain of the compared barley and wheat  
637 varieties.

<b>Species</b>	<b>Cultivar</b>	<b>Type</b>	<b>Folate content (ng g<sup>-1</sup> dm)</b>
<i>H. vulgare</i>	Mona	Hulless, spring, two-row barley	1033 ± 165 a
<i>H. vulgare</i>	Ketos	Hulled, winter, six-row barley	653 ± 65 c
<i>H. vulgare</i>	Trasimeno	Hulled, winter, two-row barley	732 ± 25 bc
<i>T. aestivum</i>	Generale	Winter common wheat	1024 ± 261 ab
<i>T. turgidum durum</i>	Colombo	Winter durum wheat	1119 ± 219 a

638 Data were analyzed after rank transformation. Values with different letters differ significantly

639 (P<0.05)

