

1 Physico-chemical and nutritional characteristics of einkorn flour cookies

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14

15 **Abstract**

16 The physico-chemical and nutritional characteristics of cookies prepared from einkorn flour and
17 their evolution during storage up to 54 days were studied. Colour, size and surface texture were
18 analysed by Image Analysis, inner texture by Bending and Penetration Test, carotenoids, tocols and
19 heat damage by HPLC on cookies prepared from refined flours of two einkorns (ID1395 and
20 Monlis) and one common wheat (Blasco), at three different times (t_0 , t_{27} and t_{54}). Einkorn cookies
21 were thinner, larger, slightly darker, with smoother surface and had higher breaking resistance than
22 the control cookies. Furthermore, they had more carotenoids and less heat damage than wheat
23 cookies, i.e. 5.0 mg/kg and 188.5 mg furosine/kg protein vs. 2.2 mg/kg and 242.4 mg furosine/kg
24 protein, respectively. Room-temperature storage under dark in sealed plastic containers led to a
25 decrease in lutein (8-17%) and furosine (20%) and an increase in hardness, especially in einkorn
26 cookies.

27 **Introduction**

28 In recent years, the interest in healthy foods prepared from cereals has focused on alternative, less
29 known wheats, such as khorasan, emmer, spelt and einkorn. Einkorn (*Triticum monococcum* L. ssp.
30 *monococcum*), a diploid ($2n=2x=14$) wheat, is probably the most promising candidate for the
31 preparation of enhanced-quality bakery products, pasta, and specialty foods because of its
32 outstanding nutritional characteristics. In fact, einkorn kernels have high contents of proteins,
33 carotenoids, tocopherols, free phenolic acids and trace minerals (Hidalgo and Brandolini 2014), as well as
34 a low lipooxygenase activity that limits antioxidant degradation during food processing (Hidalgo and
35 Brandolini 2014). Information on some einkorn-based products, such as bread, pasta, water biscuits
36 and puffed seeds, is increasing (Abdel-Aal et al. 2010; Hidalgo et al. 2018a,b; Hidalgo et al. 2016)
37 but not much is known about the technological and nutritional characteristics of einkorn sweet
38 baked goods. Corbellini et al. (1999) prepared cookies from an einkorn refined flour and noticed
39 that they were larger and thinner than those from bread wheat, while Abdel-Aal and Rabalski
40 (2013) and Abdel-Aal et al. (2010) observed that free phenolic acids augmented, and carotenoids
41 decreased after baking in whole meal cookies. Recently, Nakov et al. (2018) characterised five
42 types of cookies with increasing whole meal einkorn flour content and observed that the all-einkorn
43 ones were larger and thinner, and had more ash, protein, total polyphenols, antioxidant activity,
44 total carotenoids and β -glucans than the bread wheat ones.

45 Einkorn-enriched products fit into the health-conscious trend and its high nutritional quality can
46 play an essential role in the prevention of several diseases. However, the dearth of information is
47 hindering the development of einkorn-based functional cookies. Therefore, the aim of this paper
48 was to investigate some physico-chemical and nutritional parameters of 100% einkorn flour cookies
49 and to compare them with 100% common wheat flour cookies; additionally, a survey of the
50 evolution of these features during a 54 days storage was performed.

51

52 **2. Materials and methods**

53 *2.1. Flours*

54 *T. monococcum* advanced line ID1395, cv. Monlis, and *T. aestivum* cv. Blasco (control) were
55 cropped in 2015-16 in Sant'Angelo Lodigiano (Po plain, Italy) in a randomised complete block
56 design (RCBD) with three 10 m² plots. Standard cultural practices were followed, including limited
57 nitrogen fertilisation (80 kg/ha). The harvested kernels were stored at 5 °C until utilization. Before
58 milling, the einkorn seeds of ID1395 and Monlis were de-hulled with an Otake FC4S thresher
59 (Satake, Hiroshima, Japan); dehulling was not necessary for Blasco. After overnight tempering at
60 15% moisture (16% for Blasco), the kernels were milled with a Bona 4RB (Bona, Monza, Italy)
61 experimental mill, separating the flour fraction from bran and germ.

62

63 *2.2. Cookies*

64 The cookies were prepared according to the Official Method 10-52 (AACC 1995). The main
65 ingredients were: 40 g flour (14% moisture basis), 24 g sugar, 12 g shortening (margarine) and 1.2 g
66 non-fat dry milk, bought in a local supermarket. The cookies were baked at 205 °C for 11 min in an
67 Ovenlab rotatory oven (National MFG CO, Lincoln, Nebraska, U.S.A.). For each flour sample, two
68 independent sets of 21 cookies were obtained.

69

70 *2.3. Flour characterization*

71 The moisture content (g/100 g) of the flours was determined according to the Official Method 44-
72 15A (AACC 1995). The ash content (g/100 g) was assessed following the Official Method 08-03
73 (AACC 1995). The protein content (g/100 g DM) was quantified according to the Official Method
74 925.31 (AOAC 1995), adopting a conversion factor of 5.7. For the gliadin/glutenin ratio
75 determination, the two storage protein types were extracted as described by Pogna et al. (1990),
76 lyophilised and weighted. The evaluation of the sedimentation volume in sodium dodecyl sulphate

77 (SDS) was carried out according to Preston et al. (1982). The total starch (TS) was determined
78 using the *Total Starch Assay Kit* (Megazyme International Ireland Ltd., Bray Business Park, Bray,
79 Ireland). Fructose, glucose, maltose (reducing sugars) and sucrose were assessed by HPLC, as
80 reported by Hidalgo and Brandolini (2011). The carotenoids and the tocopherols (mg/kg DM) were
81 quantified by normal phase HPLC as detailed by Hidalgo et al. (2010) and by Hidalgo and
82 Brandolini (2010), respectively. The chemical indices that quantify the heat damage were
83 determined by HPLC: furosine was measured as reported by Hidalgo and Brandolini (2011), while
84 glucosylisomaltol (GLI) and hydroxymethylfurfural (HMF) were tested as described by Hidalgo
85 and Brandolini (2011), following Rufián-Henares et al. (2008). All the chemical analyses were
86 performed at least in duplicate ($n \geq 2$).

87

88 *2.4. Cookies characterization*

89 After cooling 1 h at room temperature, the cookies were weighted, their diameter and thickness
90 measured with a calliper, their volume computed from these data, and the specific volume
91 (volume/weight ratio) determined. The samples of each accession were then divided into three
92 groups of seven units each. A first group underwent immediate analyses (t_0) for the characterization
93 of the freshly prepared product; the remaining two groups were packaged in sealed airtight bags,
94 stored at 25 °C and analysed after 27 (t_{27}) and 54 (t_{54}) days, to evaluate cookies features evolution
95 during storage.

96 Cookies colour, size and surface texture were determined by Image Analysis (IA) on four random
97 cookies for each genotype. The samples were placed on a flatbed scanner (Epson Perfection 3170
98 Photo, Seiko Epson Corp., Japan) and covered with a black box to amplify the contrast between
99 objects and background. The images were captured at 600 dpi resolution, saved in TIFF format and
100 processed with a dedicated software (Image Pro-Plus v. 4.5.1.29, Media Cybernetics Inc, Rockville,
101 USA). The following parameters were computed: density red (R), density green (G), density blue
102 (B) and density mean for colour evaluation; area and diameter for size determination; heterogeneity

103 (HTG), *i.e.* pixels fraction that vary more than 10% from the average intensity, for surface texture
104 assessment.

105 Cookies textures (at t_0 , t_{27} and t_{54}) were examined both by the three-point fracture test (*Bending test*)
106 and by the *Penetration Test* on five random cookies for each genotype at each storage time. A
107 TA.HDplus Texture Analyser (Stable Micro Systems, Godalming, UK), controlled by the software
108 Texture Exponent TEE32 v. 3.0.4.0 (Stable Micro Systems, Godalming, UK), and equipped with a
109 500 N load-cell, was used for this purpose. For the *Bending Test* (Heavy Duty Platform-HDP/90,
110 Three Point Bending Rig-HDP/3PB) the cookies were broken by a blade moving at 2 mm/s ($n=5$).
111 The breaking force (N) was obtained from the maximum peak of the recorded force/distance curves.
112 The *Penetration Test* was performed on the five cookie halves obtained from the Bending Test,
113 carrying out measurements at four different pre-established points of the sample with a 4 mm
114 diameter probe moving at 5 mm/s. The test ended when the probe passed completely through the
115 sample. The penetration force (N) was recorded as the peak force encountered during the test, and it
116 is an index of the samples consistency.

117 Two cookies for each genotype at each storage time (t_0 , t_{27} and t_{54}) were used to determine water
118 absorption capacity, by dipping in distilled water at 25 °C for 15 and 30 s, straining for 60 s, and
119 weighting. The results are expressed as percentage of sample weight increase compared to its initial
120 weight.

121 The moisture content was determined as previously described; the water activity (a_w) was measured
122 with an AQUALAB (Decagon Devices Inc., Pullman, USA). Tocols, carotenoids and furosine
123 changes during processing were monitored, analysing some key ingredients (margarine, non-fat dry
124 milk and flours) as well as flour-less mixtures, batters and cookies at t_0 . Heat damage in the final
125 products was also assessed through HMF and GLI measurement. Lutein and heat damage indices
126 were further tested on cookies after 54 days of storage (t_{54}). All these evaluations were performed
127 twice, adopting the procedures previously described, on the fragments of the cookies used for the

128 technological tests, ground with a laboratory mill (Braun, Germany) and stored at - 20 °C until
129 analysis.

130

131 *2.5 Statistical analysis*

132 All the data were processed by analysis of variance (ANOVA), considering the samples and the
133 storage times as factors. When significant differences were found ($p \leq 0.05$), Fischer's least
134 significant difference (LSD) was computed at 95% significance level. The statistical elaboration
135 was performed using StatGraphics Plus statistical v. 5.1 (Statpoint Technologies, Inc., USA).

136

137 **3. Results and discussion**

138 *3.1. Flours*

139 Table 1 presents some characteristics of the einkorns and control flours. The ANOVA (not
140 displayed) showed significant differences ($p \leq 0.05$) among genotypes for ash, total starch and
141 protein content. Fisher's multiple comparisons test evidenced that einkorn flours had higher ash and
142 protein content and lower total starch amount than Blasco, a behaviour already observed by other
143 authors (Abdel-Aal et al. 1997; Borghi et al. 1996; Corbellini et al. 1999; Løje et al. 2003).

144 The glutenin/gliadin ratio of the einkorn accessions (1.73 for ID1395 and 1.93 for Monlis) was
145 higher than that of Blasco (1.50), as observed also by Abdel-Aal et al. (1995); this difference could
146 contribute to explain the diversity in gluten strength and elasticity between *T. monococcum* and *T.*
147 *aestivum* reported by Borghi et al. (1996) and Corbellini et al. (1999), as glutenins mainly influence
148 gluten elasticity and toughness, while gliadins affect viscosity and extensibility (Wrigley et al.
149 2006). A broad variation of the SDS sedimentation among the samples was observed, in particular
150 between the two einkorn accessions further confirming the results of other researchers (Borghi et al.
151 1996; Corbellini et al. 1999). Blasco SDS volume, in fact, was high (73 mL) but lower than that of
152 Monlis (92 mL); on the other hand, ID 1395 had a very low value (15 mL). SDS-sedimentation
153 volume is mainly controlled by the quantity and quality of protein: superior volumes generally

154 indicate higher proportions of glutenin in the flour, which make the system more elastic. Cookies
155 production do not generally require an extensive gluten development, and a high protein content is
156 usually undesirable.

157 3.2. Cookies

158 3.2.1. Physical characteristics

159 In the design of new products, parameters such as colour, texture and volume which directly
160 influence consumer acceptance, anticipating specific tactile perceptions (Jianshe 2007), and are of
161 extreme importance. Cookies weight, diameter, thickness, moisture content, water activity, colour,
162 surface texture, and hardness were assessed 1 h after cooling at room temperature (t_0); the results
163 are reported in Table 2. The ANOVA (*not shown*) highlighted significant differences ($p < 0.05$)
164 among the samples for all the investigated parameters.

165 The einkorn cookies were heavier, thinner, larger, and with a higher volume in comparison to those
166 from Blasco, in accordance with data from Corbellini et al. (1999) and Nakov et al. (2018). The
167 largest diameter and the lowest thickness were found for ID1395, which was characterised by the
168 lowest SDS value (Table 1). Low SDS volumes indicate lower proportion of glutenin in the flour,
169 which in turn makes the dough less elastic and thus increases the cookie spread. Cookie spread,
170 represented by the diameter-to-thickness ratio, was highest for ID1395. Of course, many other
171 factors (e.g. gluten quantity and quality) contribute to these results. For instance, the gluten content
172 of ID1395 was not measurable because gluten could not even be formed in the Glutomatic (Perten,
173 Hägersten, Sweden) testing machine; on the contrary, gluten content was 11.3 ± 0.07 g/100 g DM
174 and to 19.6 ± 0.21 g/100 g DM for Blasco and Monlis, respectively.

175 The specific volume, related to the degree of compactness of the internal structure of the product,
176 higher for Blasco, thus suggesting a more compact, texture of the einkorn cookies. This may be
177 partially explained by the different particle size distribution of the flours, which show a prevalence
178 of smaller particles in the einkorn accessions, and a prevalence of coarser particles in the wheat

179 flour (diameter < 125 μ m: Blasco, 51.7%; ID1395, 76.1%; Monlis, 86.2%), as well as by the higher
180 protein content of einkorns.

181 The cookies from Blasco showed the highest R, G, B and density values, indicating a lighter colour
182 due to the different nature of the flours. Cookies images were also used to determine the surface
183 heterogeneity (HTG) of the products, a parameter defined as the fraction of pixels whose intensity
184 value deviates more than 10% compared to the average intensity of the entire image, and that ranges
185 from 0 (homogeneous, smooth surface) to 1 (heterogeneous, rough surface) (Fongaro and Kvaal,
186 2013). Blasco cookies were characterised by a higher HTG, compared to the cookies obtained from
187 the einkorns accessions, highlighting a more rough and non-homogeneous surface.

188 The results of the *Bending Test* and of the *Penetration Test* are presented in Figure 1. As for the
189 *Bending Test*, in general, the einkorn cookies showed a significantly higher resistance to breaking
190 than the Blasco samples despite their lower thickness, highlighting a more compact structure and
191 confirming what already hinted by the specific volume results. ID1395, characterised by the lowest
192 thickness and the largest diameter, had the greatest resistance to breaking (breaking force,
193 37.3 \pm 2.87 N), while Blasco was the most easily fracturable (breaking force, 26.8 \pm 2.50 N). As for
194 the *Penetration Test*, performed to obtain further information on cookies consistency, no significant
195 differences among the measurements carried out at the four different pre-established points of the
196 samples were observed, therefore all the results obtained (five cookies, four measurements each:
197 n=20) were grouped for data elaboration. ID1395 was the most consistent and hard (penetration
198 force, 38.7 \pm 8.86 N), while Monlis had the lowest resistance to penetration (penetration force,
199 27.1 \pm 7.81 N), comparable to Blasco (penetration force, 31.5 \pm 7.17 N). This may be due to the
200 higher levels of moisture and a_w of Monlis products. Probably, cookies moisture and a_w play a
201 major role on the deformations adopted in the *Penetration Test* (Monlis higher levels of moisture
202 and a_w), while specific volume and compactness seem to have a major influence on the
203 deformations during the *Bending Test*. This information is very important for the modulation of the
204 features of the end product in relation to product specifications and consumers expectations.

205 The water absorption test showed a relevant increase in cookies weight after 15 s dipping in water
206 (93.8%±3.74, 85.6%±5.95 and 69.4%±5.21 for Blasco, Monlis and ID1395, respectively). However,
207 after 30 s the absorption was lower (88.1±3.66, 75.8±5.95 and 59.3±7.95, respectively) as the
208 integrity of the samples started waning because of loss of material in water. The lower absorption
209 capacity shown by the einkorn cookies is probably linked to their greater compactness, which delays
210 the access of water, and possibly to their higher protein content, that favours the formation of a more
211 compact protein network during baking, which in turns slows water absorption.

212 Cookies features were also evaluated during storage up to 54 days. The ANOVA (*not shown*)
213 indicated the existence of significant effects for cookie type and storage time, as well as for their
214 interaction, for the investigated parameters. The only exception was weight, where storage time did
215 not have any significant influence. Moisture and a_w did not vary greatly from t_0 to t_{54} , and only a
216 limited reduction was observed in einkorn samples, particularly in Monlis cookies. Changes were
217 more limited in Blasco, in comparison to the einkorn cookies. As for the *Bending Test*, einkorn
218 cookies, which were more resistant to fracture at t_0 than those from Blasco, showed different
219 behaviours during storage: while ID1395 cookies softened to breaking forces comparable to Blasco,
220 the hardness did not change significantly over time for Monlis. As for the *Penetration Test*, Monlis
221 and ID1395 cookies recorded an increase in the penetration force reaching, at the end of the storage,
222 values higher than Blasco cookies. These increases were present for both einkorn cookies already
223 after 27 storage days, but only for Monlis continued up to the last sampling time (54 days).

224 3.2.2. Chemical characteristics

225 3.2.2.1. Carotenoids and tocols

226 Table 3 shows the content in tocols and carotenoids of the flour-less mixture as well as of the flour,
227 batter and cookies from Blasco, ID1395 and Monlis; the values of margarine and non-fat dry milk
228 are reported in Supplementary Table 1. The profile in tocols and carotenoids of Blasco and the two
229 einkorn accessions was like the data reported by Hidalgo et al. (2010) and by Hidalgo and Brandolini
230 (2010). Interestingly, in the three flours were present four compounds, β -tocotrienol, β -

231 cryptoxanthin, lutein and zeaxanthin, not found in margarine and non-fat dry milk. In the batter, the
232 composition well reflected the contribution of the different ingredients. The high amounts of all the
233 tocopherols (except β -tocopherol) and $(\alpha + \beta)$ -carotene in the flour-less mixture minimised the differences
234 between the accessions in relation to these compounds. However, the higher concentration in total
235 tocopherols and total carotenoids of Monlis flour was carried on in the batter and finally resulted in
236 cookies with a significantly higher content.

237 The influence of manufacturing on the antioxidants coming from the flour will be discussed only for
238 lutein and β -tocopherol, because β -cryptoxanthin and zeaxanthin were present in minimal
239 concentrations. To better differentiate the concentration in the mixture (flour, margarine, non-fat dry
240 milk, sugar and raising agents) from the kneading effect, the theoretical levels in lutein and β -
241 tocopherol were computed, as their decrease from flour to batter is mainly due to the dilution effect
242 for the addition of the flour-less mixture. The percentage of degradation between the theoretical
243 value of the mixture and the batter on average was 29.2% for lutein and 13.8% for β -tocopherol.
244 Lower lutein degradation (11.7% on average) but higher β -tocopherol degradation (28.2%) during
245 kneading were observed by Hidalgo et al. (2010) in water biscuits prepared only with flour and
246 water. These differences are probably related to the presence, in cookies, of other ingredients, which
247 modify the protective mechanisms of antioxidants.

248 The degradation from batter to cookie was 20.9%, for lutein and 5.5% for β -tocopherol on average;
249 similar results were reported by Hidalgo et al. (2010) and by Hidalgo and Brandolini (2010) for
250 water biscuits. Despite the antioxidant compounds losses during processing, it is important to
251 emphasize the significant lutein presence in einkorn cookies compared to bread wheat cookies (on
252 average, 2.37 vs. 0.17 mg/kg DM), a difference that visually translates into an appealing yellow
253 colour of the finished product. On the other hand, the difference in β -tocopherol was minimal (on
254 average, 7.2 vs. 6.0 mg/kg DM, respectively).

255 During storage, the lutein content in ID1395 and Monlis cookies decreased 15% and 17% compared
256 to the samples at t_0 , while in Blasco the variation was only 8%, probably because of the very low

257 initial level. The cookies were stored at atmospheric pressure, in the dark, and in sealed plastic
258 containers to prevent air from entering; the limited degradation observed may be associated with
259 non-enzymatic oxidation phenomena triggered by the presence of residual oxygen inside the
260 package.

261 3.2.1.2. Heat damage

262 Furosine concentration (Figure 2) was very low in the flours (4.5-9.3 mg/kg protein), similarly to
263 the data reported by Guerra-Hernández et al. (1999) and Hidalgo and Brandolini (2011); the
264 slightly higher initial furosine content of Blasco flour compared to the einkorns might be linked to
265 the major hardness of wheat kernels, which need higher energy for milling and generate superior
266 grinding temperatures. Furosine concentration was moderately higher in both mixture and batter,
267 owing to the presence of the nonfat dry milk, which contains a lot of furosine (639.7 mg/100 g
268 protein), but grew considerably during baking, reaching 242.4 mg/kg protein in Blasco cookies vs.
269 188.5 mg/kg protein, on average, in einkorn cookies. The major heat damage observed in Blasco
270 cookies was partially due to the higher reducing sugars content (Supplementary Table 2) in Blasco
271 flour and batter (0.17 and 0.62 g/100 g DM, respectively) compared to ID1395 (0.07 and 0.40 g/100
272 g DM) and Monlis (0.07 and 0.52 g/100 g DM). The addition of sucrose (a non-reducing sugar) to
273 the batter did not significantly influence the development of furosine because, to participate to the
274 Maillard reaction, its conversion into glucose and fructose is needed (Gökmen et al., 2008).
275 Furosine content in cookies was higher than those (15-20 and 35-45 g/100 g protein) reported by
276 Hidalgo and Brandolini (2011) and those (42 and 106 g/100 g protein) observed by Hidalgo et al.
277 (2018a) for einkorn and bread wheat water biscuits, respectively; the difference could be due to the
278 presence of nonfat dry milk which, in addition to providing a certain amount of furosine, enriched
279 the mixture of reducing sugars (lactose) and proteins with lysine residues, ideal reagents for the
280 Maillard reaction and leading to rapid formation of the Amadori compounds (Erbersdobler and
281 Somoza 2007). However, furosine concentration in the cookies was still in the lower end of the

282 variation (25-982 mg/100 g protein; average: 362 mg/100 g protein) of sugar-containing
283 commercial biscuits (Rada-Mendoza et al. 2004).

284 The analysis explored also the presence of some intermediate compounds of the Maillard reaction,
285 but no detectable quantities of HMF were found, while GLI was observed at very low levels (on
286 average, 1.2 mg/kg DM). Hence, the HMF concentration was lower and the GLI content was
287 similar to the values (1.1-3.9 and 1.3-1.4 mg/kg DM, respectively) presented by Hidalgo et al.
288 (2018b) for whole-meal einkorn and bread wheat water biscuits; additionally, GLI was within the
289 variation (not detectable to 4.0 mg/kg DM) reported by Hidalgo and Brandolini (2011) for refined
290 flour water biscuits. Additionally, the HMF was inferior to the results (7.4 mg/kg) observed by
291 Kocadağlı and Gömen (2016) in their control cookies, while the GLI was lower than the values (2.7
292 to 9.5 mg/kg DM) found by Rufián-Henares et al. (2008) in cookies obtained from wheat flour and
293 other cereals, baked at 200 °C for 20 min. High HMF and GLI concentrations are reported for
294 cookies baked under more exacting temperatures, as Ramírez-Jiménez et al. (2000) found an
295 average HMF value of 15.6 mg/kg DM after baking at 180 °C for 90 min; interestingly, Ait Ameer
296 et al. (2008) found that inferior cooking temperatures resulted in lower HMF content but also that,
297 as cooking advanced (typically after 8 min), this compound decreased as a result of its volatilization
298 and degradation in other molecules, such as furaldehyde and methylfurfural.

299 The decrease in furosine content in cookies after storage (Figure 2) was about 20%, and is similar to
300 the results reported by Bosch et al. (2008) in baby food based on cereals containing milk and stored
301 at 25 °C. Concerning the other two indices, a small increase in GLI (on average, 1.43 mg/kg DM)
302 was found, while HMF was just above the detection limit (1.39 mg/kg DM). Therefore, the furosine
303 reduction in the initial conservation phases may be somehow linked to a partial change to HMF or
304 GLI.

305

306 **4. Conclusions**

307 Our results show that einkorn is very suitable to produce high-nutritional-value cookies. Einkorn
308 cookies were thinner, larger, slightly darker, with smoother surface and better breaking resistance,
309 had more carotenoids and less heat damage than the common wheat control. During room-
310 temperature storage lutein (8-17%) and furosine (20%) decreased, while hardness increased,
311 especially in einkorn cookies.

312

313 **Conflict of interests**

314

315 The authors declare no conflict of interests

316

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393

394

395 **Captions to Figures**

396

397 **Figure 1.** Variation of breaking force and penetration force during the storage ($t_0=0$ days; $t_{27}=27$
398 days; $t_{54}=54$ days) of cookies prepared from common wheat (Blasco) and einkorn (ID1395 and
399 Monlis) flours.

400

401 **Figure 2.** Content of furosine in flour, mixture, batter, freshly-baked cookies (t_0) and cookies stored
402 for 54 days at 25 °C (t_{54}), prepared from common wheat (Blasco) and einkorn (ID1395 and Monlis)
403 flours.

404

405 Table 1. Mean values (\pm standard error) of moisture (g/100 g), ash, protein, total starch content
 406 (g/100 g DM), SDS sedimentation volume (mL), and glutenin/gliadin ratio of flours from wheat
 407 (Blasco) and einkorn (Monlis and ID1395) flours.
 408

	Blasco	ID1395	Monlis
Moisture	13.32 \pm 0.05	12.14 \pm 0.06	14.07 \pm 0.03
Ash	0.55 ^c \pm 0.013	0.66 ^b \pm 0.023	0.75 ^a \pm 0.018
Protein	13.2 ^c \pm 0.01	18.4 ^b \pm 0.08	18.4 ^a \pm 0.01
Total starch	86.0 ^a \pm 0.21	77.7 ^b \pm 0.21	75.6 ^c \pm 0.07
SDS sedimentation volume	73.0 ^b \pm 0.30	15.0 ^c \pm 0.11	92.0 ^a \pm 3.00
Glutenin/gliadin ratio	1.50	1.73	1.93

409
 410 Different letters in a row indicate significant LSD differences ($p \leq 0.05$) among samples
 411

412 Table 2. Mean value (\pm standard error) of weight, diameter, thickness, volume, specific volume,
 413 moisture, water activity (a_w), colorimetric indices (R, G, B and density) and heterogeneity (HTG) of
 414 cookies from wheat (Blasco) and einkorn (ID1395 and Monlis) flours.
 415

	Blasco	ID1395	Monlis
Weight (g)	21.83 ^c \pm 0.56	23.59 ^b \pm 0.58	24.80 ^a \pm 1.10
Diameter (cm)	8.52 ^c \pm 0.05	9.27 ^a \pm 0.07	8.64 ^b \pm 0.17
Thickness (cm)	0.69 ^a \pm 0.03	0.54 ^c \pm 0.02	0.63 ^b \pm 0.04
Volume (cm ³)	35.00 ^b \pm 0.53	36.64 ^a \pm 0.25	36.92 ^a \pm 0.72
Specific volume (cm ³ /g)	1.61 ^c \pm 0.02	1.55 ^b \pm 0.01	1.49 ^a \pm 0.03
Moisture (g/100 g)	5.95 ^b \pm 0.10	5.92 ^b \pm 0.07	6.7 ^a \pm 0.12
a_w	0.45 ^b \pm 0.01	0.46 ^b \pm 0.01	0.50 ^a \pm 0.01
R	177.8 ^a \pm 2.77	165.1 ^b \pm 1.14	168.1 ^b \pm 2.43
G	136.6 ^a \pm 3.05	129.6 ^a \pm 2.33	129.2 ^a \pm 3.44
B	70.1 ^a \pm 2.08	61.5 ^b \pm 1.30	61.6 ^b \pm 1.93
Density	128.1 ^a \pm 2.60	118.7 ^b \pm 1.36	119.6 ^b \pm 2.57
HTG	0.20 ^a \pm 0.02	0.15 ^b \pm 0.01	0.14 ^b \pm 0.01

416
 417 Different letters in a row indicate significant LSD differences ($p \leq 0.05$) among samples
 418

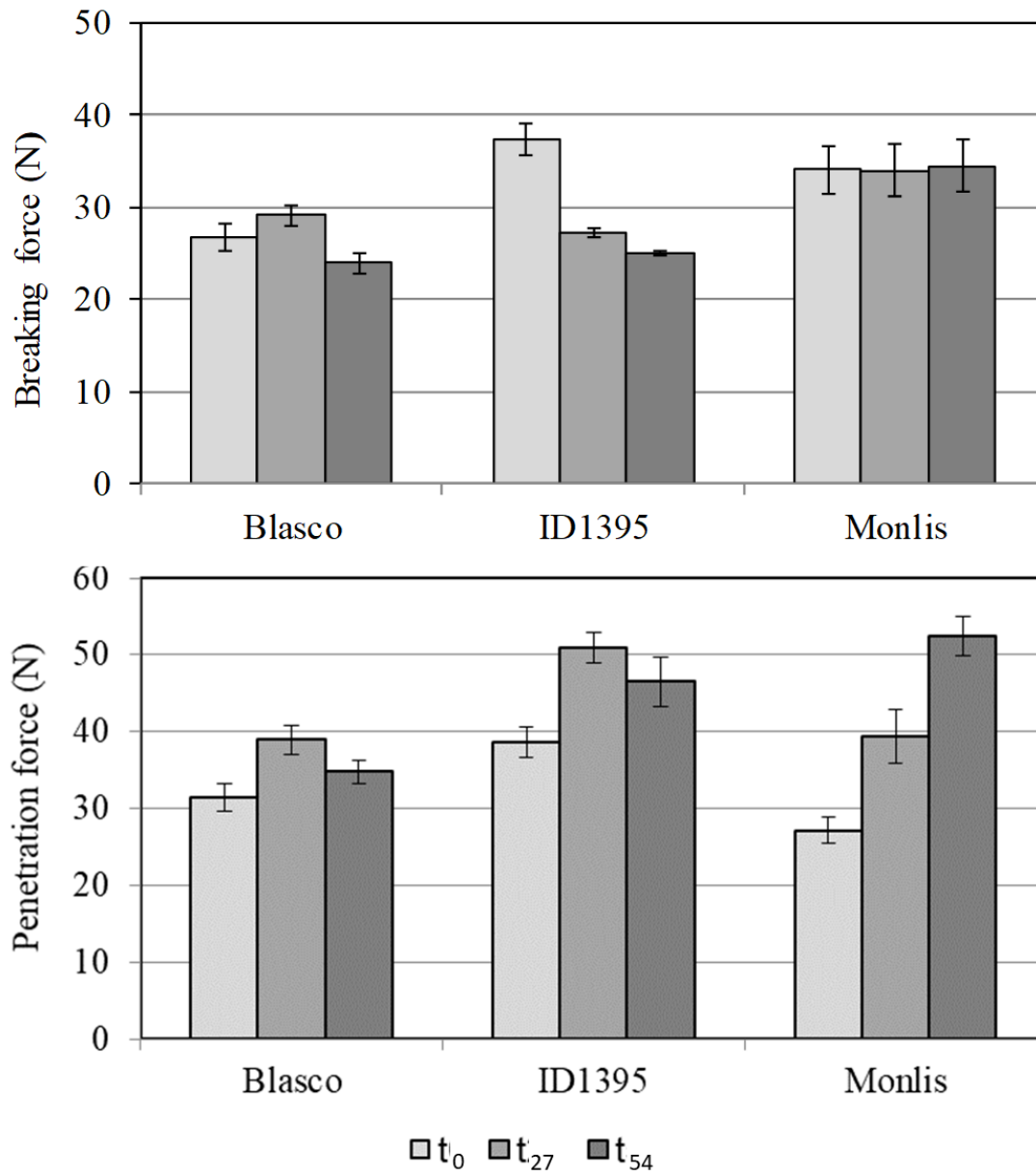
419 Table 3. Mean value (\pm standard error) of tocopherols and carotenoids (mg/kg DM) content in flour-less mix, flour, batter and freshly-baked cookies from
 420 wheat (Blasco) and einkorn (ID1395 and Monlis).
 421

	Flour-less	Blasco			ID1395			Monlis		
	mixture	Flour	Batter	Cookie	Flour	Batter	Cookie	Flour	Batter	Cookie
α -tocopherol	31.6 \pm 0.34	2.0 \pm 0.24	17.6 \pm 0.09	16.8 \pm 0.06	2.1 \pm 0.18	14.9 \pm 0.09	10.6 \pm 0.15	4.6 \pm 0.35	18.5 \pm 1.17	17.9 \pm 0.22
α -tocotrienol	19.0 \pm 0.40	1.0 \pm 0.06	10.4 \pm 0.16	9.8 \pm 0.05	2.3 \pm 0.03	10.1 \pm 0.15	10.4 \pm 0.20	6.1 \pm 0.02	13.7 \pm 0.93	12.9 \pm 0.28
β -tocopherol	4.0 \pm 0.16	1.2 \pm 0.05	2.2 \pm 0.04	2.2 \pm 0.03	1.6 \pm 0.31	2.2 \pm 0.17	2.0 \pm 0.17	1.4 \pm 0.02	2.3 \pm 0.05	1.9 \pm 0.03
β -tocotrienol	nd	12.7 \pm 0.04	6.3 \pm 0.02	6.0 \pm 0.00	18.3 \pm 0.59	7.5 \pm 0.26	7.4 \pm 0.06	18.7 \pm 0.23	7.8 \pm 0.10	7.0 \pm 0.22
γ -tocopherol	64.3 \pm 1.13	nd	29.5 \pm 0.30	27.5 \pm 0.42	nd	27.4 \pm 0.47	28.0 \pm 0.51	nd	30.2 \pm 0.89	29.3 \pm 0.28
γ -tocotrienol	28.7 \pm 1.36	nd	13.4 \pm 0.27	12.3 \pm 0.20	nd	14.0 \pm 0.05	13.9 \pm 0.13	nd	14.5 \pm 0.25	13.3 \pm 0.36
δ -tocopherol	19.1 \pm 0.54	nd	9.1 \pm 0.06	8.2 \pm 0.01	nd	9.4 \pm 0.05	9.2 \pm 0.17	nd	9.7 \pm 0.25	9.1 \pm 0.05
δ -tocotrienol	6.7 \pm 0.42	nd	3.2 \pm 0.03	3.0 \pm 0.16	nd	3.6 \pm 0.13	3.5 \pm 0.02	nd	3.6 \pm 0.15	3.2 \pm 0.21
Total tocopherols	173.4 \pm 2.55	16.9 \pm 0.32	91.8 \pm 0.93	85.9 \pm 0.30	24.3 \pm 1.12	89.1 \pm 0.61	85.2 \pm 1.37	30.7 \pm 0.08	100.4 \pm 3.69	94.6 \pm 1.58
$(\alpha+\beta)$ -carotene	4.0 \pm 0.13	nd	1.8 \pm 0.04	2.1 \pm 0.33	0.7 \pm 0.03	2.1 \pm 0.01	2.1 \pm 0.02	1.1 \pm 0.01	2.5 \pm 0.01	2.4 \pm 0.04
β -cryptoxanthin	nd	0.06 \pm 0.005	nd	nd	0.07 \pm 0.008	0.03 \pm 0.002	0.03 \pm 0.001	0.10 \pm 0.020	0.04 \pm 0.003	0.05 \pm 0.008
Lutein	nd	0.6 \pm 0.02	0.2 \pm 0.02	0.2 \pm 0.04	6.7 \pm 0.42	2.4 \pm 0.05	1.8 \pm 0.02	9.7 \pm 0.09	3.6 \pm 0.03	2.9 \pm 0.11
Zeaxanthin	nd	0.04 \pm 0.001	nd	nd	0.18 \pm 0.028	0.06 \pm 0.002	0.04 \pm 0.004	0.18 \pm 0.004	0.07 \pm 0.006	0.06 \pm 0.002
Total carotenoids		0.7 \pm 0.02	2.0 \pm 0.06	2.2 \pm 0.38	7.6 \pm 0.48	4.6 \pm 0.06	4.0 \pm 0.04	11.2 \pm 0.10	6.3 \pm 0.04	5.4 \pm 0.15

422

423 nd: not detected

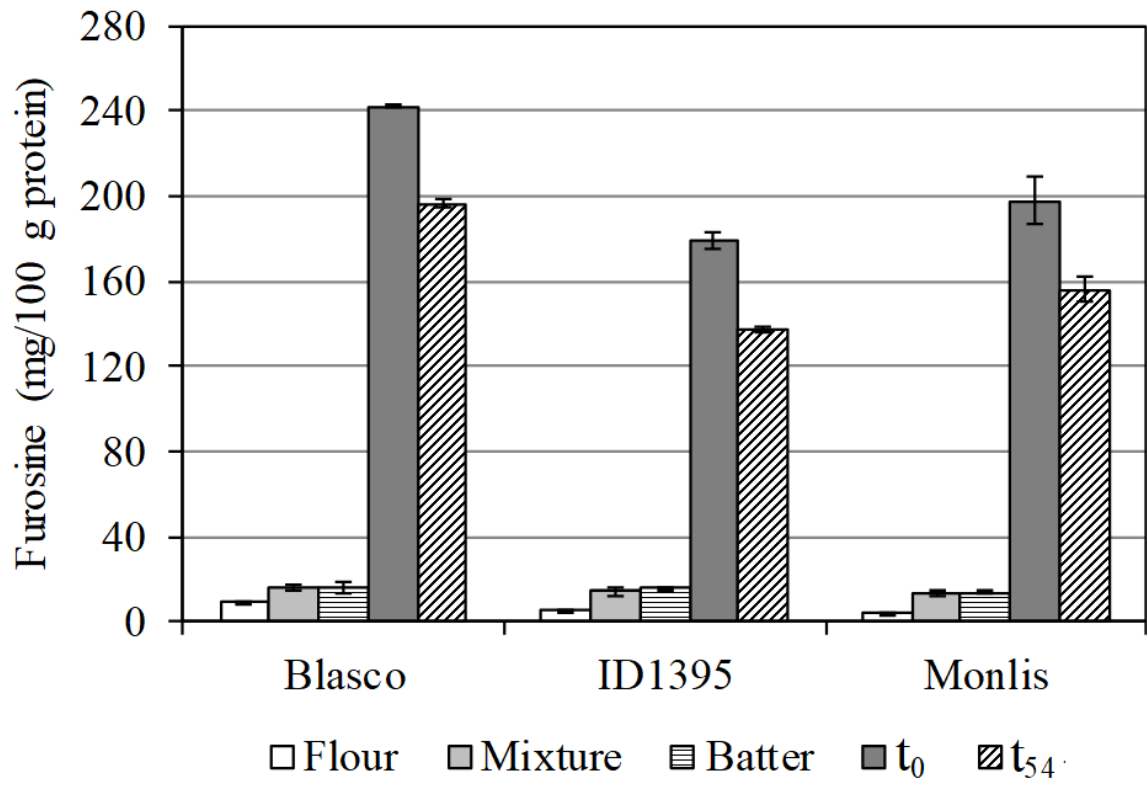
424



425

426 Figure 1

427



428

429 Figure 2

430

431 Supplementary Table 1. Mean content (\pm standard error) of tocols, α + β -carotene (mg/kg DM), and
 432 furosine (mg/100 g protein) in margarine and non-fat dry milk used in cookie preparation.

	Margarine	Non-fat dry milk
435 α -tocopherol	85.6 \pm 1.59	55.0 \pm 0.99
436 α -tocotrienol	63.2 \pm 2.81	8.7 \pm 0.24
437 β -tocopherol	7.2 \pm 0.13	1.3 \pm 0.06
438 γ -tocopherol	201.4 \pm 1.85	27.1 \pm 0.70
439 γ -tocotrienol	84.5 \pm 0.74	8.5 \pm 0.43
440 δ -tocopherol	62.6 \pm 0.75	0.5 \pm 0.02
441 δ -tocotrienol	20.3 \pm 0.28	1.6 \pm 0.06
442 Total tocols	524.9 \pm 0.91	102.5 \pm 0.89
443 α + β -carotene	12.3 \pm 1.02	3.6 \pm 0.21
444 Furosine	nd	639.7 \pm 14.56

446 nd= not detected

447
 448
 449 Supplementary Table 2. Fructose, glucose, maltose, total reducing sugars and sucrose content
 450 (g/100 g DM) of flours, batters and cookies from bread wheat (Blasco) and einkorn (Monlis and
 451 ID1395)

	Blasco			ID1395			Monlis		
	Flour	Batter	Cookie	Flour	Batter	Cookie	Flour	Batter	Cookie
Fructose	0.05	0.07	0.12	0.05	0.05	0.09	0.05	0.07	0.08
Glucose	0.06	0.11	0.06	0.02	0.03	0.05	0.02	0.07	0.05
Maltose	0.06	0.44	0.28	nd	0.32	0.19	nd	0.38	nd
Total reducing sugars	0.17	0.62	0.46	0.07	0.40	0.33	0.07	0.52	0.13
Sucrose	0.47	24.79	24.31	0.30	25.21	24.50	0.46	24.40	24.00

452 nd= not detectable