Physico-chemical and nutritional characteristics of einkorn flour cookies

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Abstract

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

Introduction

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

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

2. Materials and methods

2.1. Flours

T. monococcum advanced line ID1395, cv. Monlis, and *T. aestivum* cv. Blasco (control) were cropped in 2015-16 in Sant'Angelo Lodigiano (Po plain, Italy) in a randomised complete block design (RCBD) with three 10 m^2 plots. Standard cultural practices were followed, including limited nitrogen fertilisation (80 kg/ha). The harvested kernels were stored at 5 °C until utilization. Before milling, the einkorn seeds of ID1395 and Monlis were de-hulled with an Otake FC4S thresher (Satake, Hiroshima, Japan); dehulling was not necessary for Blasco. After overnight tempering at 15% moisture (16% for Blasco), the kernels were milled with a Bona 4RB (Bona, Monza, Italy) experimental mill, separating the flour fraction from bran and germ.

2.2. Cookies

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

2.3. Flour characterization

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

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

2.4. Cookies characterization

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

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

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

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

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

The moisture content was determined as previously described; the water activity (a_w) was measured with an AQUALAB (Decagon Devices Inc., Pullman, USA). Tocols, carotenoids and furosine changes during processing were monitored, analysing some key ingredients (margarine, non-fat dry milk and flours) as well as flour-less mixtures, batters and cookies at t_0. Heat damage in the final products was also assessed through HMF and GLI measurement. Lutein and heat damage indices were further tested on cookies after 54 days of storage (t_{54}). All these evaluations were performed twice, adopting the procedures previously described, on the fragments of the cookies used for the technological tests, ground with a laboratory mill (Braun, Germany) and stored at - 20 °C until analysis.

2.5 Statistical analysis

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

3. Results and discussion

3.1. Flours

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

The glutenin/gliadin ratio of the einkorn accessions (1.73 for ID1395 and 1.93 for Monlis) was higher than that of Blasco (1.50), as observed also by Abdel-Aal et al. (1995); this difference could contribute to explain the diversity in gluten strength and elasticity between *T. monococcum* and *T. aestivum* reported by Borghi et al. (1996) and Corbellini et al. (1999), as glutenins mainly influence gluten elasticity and toughness, while gliadins affect viscosity and extensibility (Wrigley et al. 2006). A broad variation of the SDS sedimentation among the samples was observed, in particular between the two einkorn accessions further confirming the results of other researchers (Borghi et al. 1996; Corbellini et al. 1999). Blasco SDS volume, in fact, was high (73 mL) but lower than that of Monlis (92 mL); on the other hand, ID 1395 had a very low value (15 mL). SDS-sedimentation volume is mainly controlled by the quantity and quality of protein: superior volumes generally indicate higher proportions of glutenin in the flour, which make the system more elastic. Cookies production do not generally require an extensive gluten development, and a high protein content is usually undesirable.

3.2. Cookies

3.2.1. Physical characteristics

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

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

The specific volume, related to the degree of compactness of the internal structure of the product, higher for Blasco, thus suggesting a more compact, texture of the einkorn cookies. This may be partially explained by the different particle size distribution of the flours, which show a prevalence of smaller particles in the einkorn accessions, and a prevalence of coarser particles in the wheat flour (diameter < 125μm: Blasco, 51.7%; ID1395, 76.1%; Monlis, 86.2%), as well as by the higher protein content of einkorns.

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

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

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

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

3.2.2. Chemical characteristics

3.2.2.1. Carotenoids and tocols

Table 3 shows the content in tocols and carotenoids of the flour-less mixture as well as of the flour, batter and cookies from Blasco, ID1395 and Monlis; the values of margarine and non-fat dry milk are reported in Supplementary Table 1. The profile in tocols and carotenoids of Blasco and the two einkorn accessions was like the data reported by Hidalgo et al. (2010) and by Hidalgo and Brandolini (2010). Interestingly, in the three flours were present four compounds, β-tocotrienol, β-cryptoxanthin, lutein and zeaxanthin, not found in margarine and non-fat dry milk. In the batter, the composition well reflected the contribution of the different ingredients. The high amounts of all the tocols (except β-tocotrienol) and (α + β)-carotene in the flour-less mixture minimised the differences between the accessions in relation to these compounds. However, the higher concentration in total tocols and total carotenoids of Monlis flour was carried on in the batter and finally resulted in cookies with a significantly higher content.

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

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

During storage, the lutein content in ID1395 and Monlis cookies decreased 15% and 17% compared to the samples at t_0, while in Blasco the variation was only 8%, probably because of the very low initial level. The cookies were stored at atmospheric pressure, in the dark, and in sealed plastic containers to prevent air from entering; the limited degradation observed may be associated with non-enzymatic oxidation phenomena triggered by the presence of residual oxygen inside the package.

3.2.1.2. Heat damage

Furosine concentration (Figure 2) was very low in the flours (4.5-9.3 mg/kg protein), similarly to the data reported by Guerra-Hernández et al. (1999) and Hidalgo and Brandolini (2011); the slightly higher initial furosine content of Blasco flour compared to the einkorns might be linked to the major hardness of wheat kernels, which need higher energy for milling and generate superior grinding temperatures. Furosine concentration was moderately higher in both mixture and batter, owing to the presence of the nonfat dry milk, which contains a lot of furosine (639.7 mg/100 g protein), but grew considerably during baking, reaching 242.4 mg/kg protein in Blasco cookies vs. 188.5 mg/kg protein, on average, in einkorn cookies. The major heat damage observed in Blasco cookies was partially due to the higher reducing sugars content (Supplementary Table 2) in Blasco flour and batter (0.17 and 0.62 g/100 g DM, respectively) compared to ID1395 (0.07 and 0.40 g/100 g DM) and Monlis (0.07 and 0.52 g/100 g DM). The addition of sucrose (a non-reducing sugar) to the batter did not significantly influence the development of furosine because, to participate to the Maillard reaction, its conversion into glucose and fructose is needed (Gökmen et al., 2008). Furosine content in cookies was higher than those (15-20 and 35-45 g/100 g protein) reported by Hidalgo and Brandolini (2011) and those (42 and 106 g/100 g protein) observed by Hidalgo et al. (2018a) for einkorn and bread wheat water biscuits, respectively; the difference could be due to the presence of nonfat dry milk which, in addition to providing a certain amount of furosine, enriched the mixture of reducing sugars (lactose) and proteins with lysine residues, ideal reagents for the Maillard reaction and leading to rapid formation of the Amadori compounds (Erbersdobler and Somoza 2007). However, furosine concentration in the cookies was still in the lower end of the variation (25-982 mg/100 g protein; average: 362 mg/100 g protein) of sugar-containing commercial biscuits (Rada-Mendoza et al. 2004).

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

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

4. Conclusions

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

Conflict of interests

The authors declare no conflict of interests

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Captions to Figures

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

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

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

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

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

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

	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	$0.20^{a} \pm 0.02$	$0.15^{b} \pm 0.01$	$0.14^{b} \pm 0.01$

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

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

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

nd: not detected

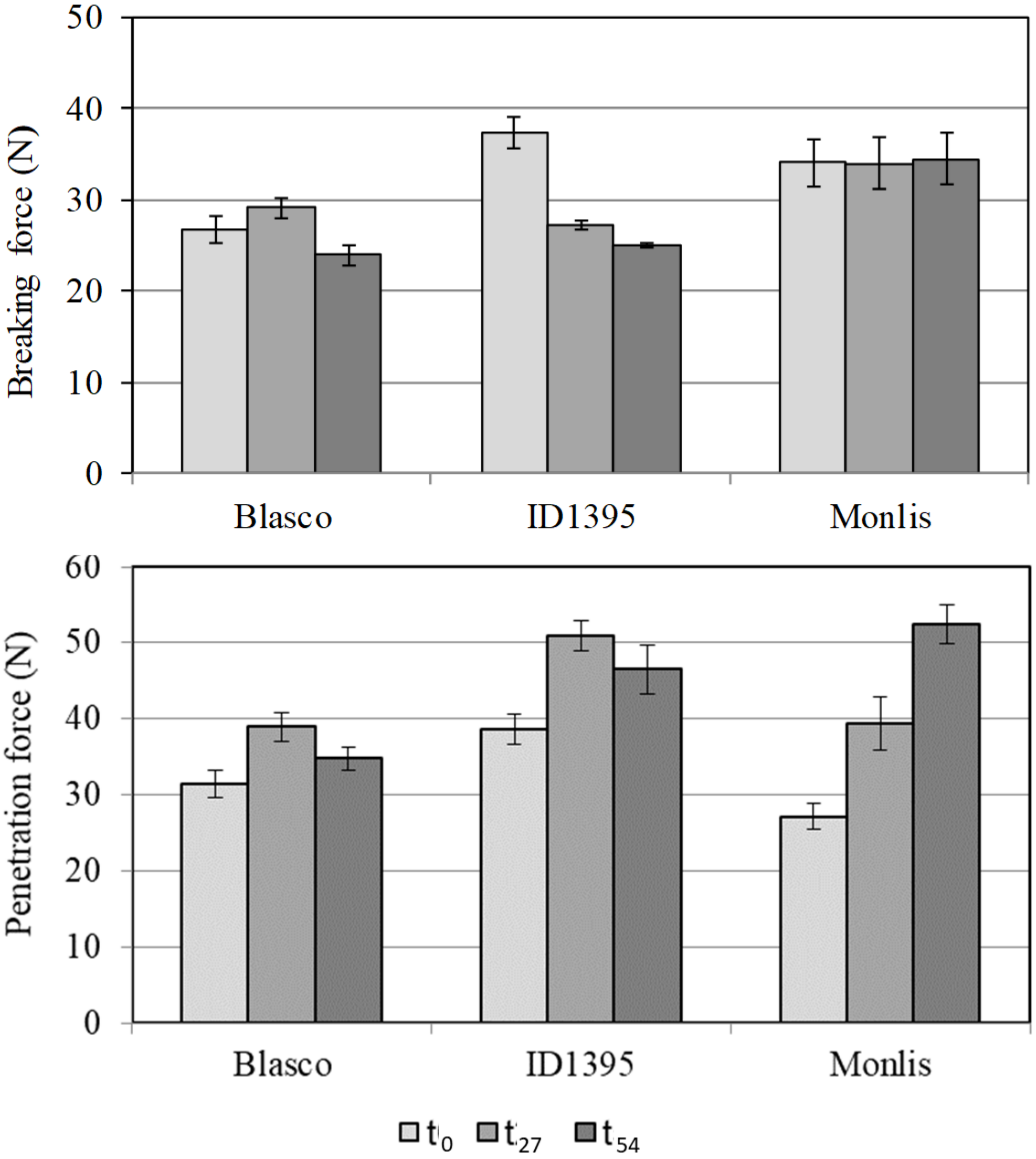
Breaking force (N)
50
40
30
20
10
0
Blasco
ID1395
Monlis
Penetration force (N)
60
50
40
30
20
10
0
Blasco
ID1395
Monlis
t_0
t_{27}
t_{54}

Figure 1

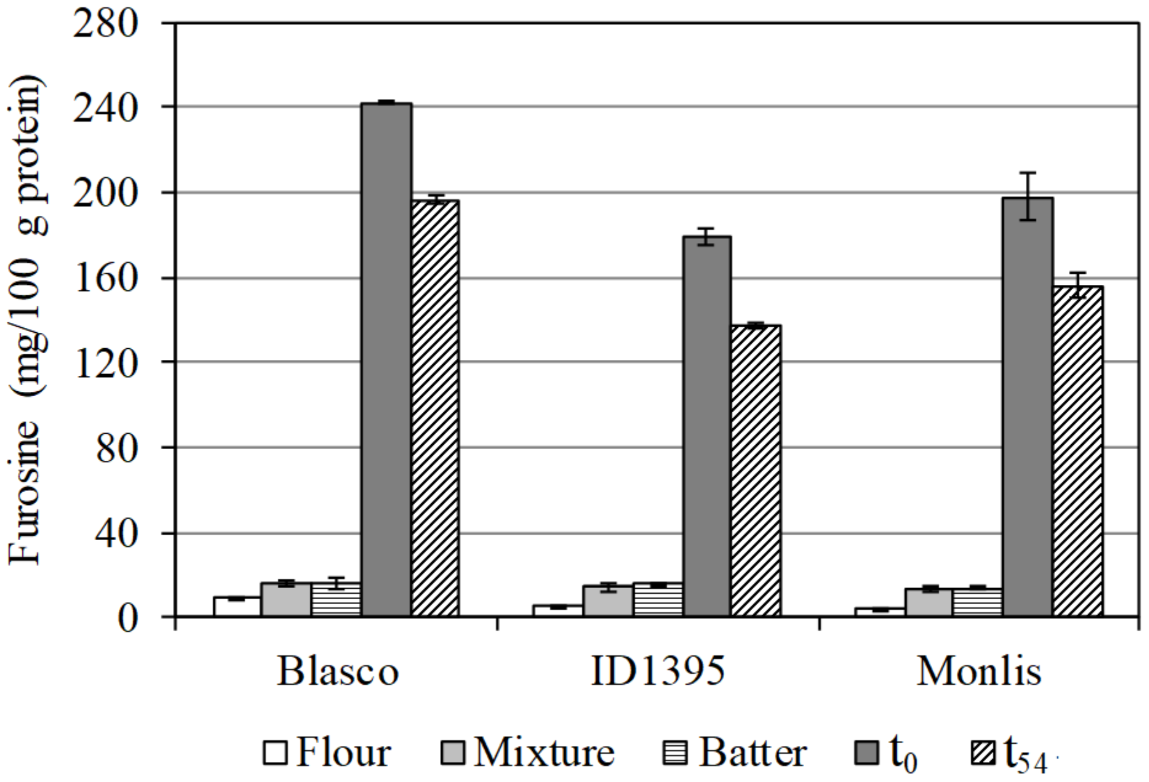
Furosine (mg/100 g protein)
0
40
80
120
160
200
240
280
Blasco
ID1395
Monlis
Flour
Mixture
Batter
t0
t54

Figure 2

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

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

nd= not detected

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

nd= not detectable