Influence of water biscuit processing and kernel puffing on the phenolic acid content and the antioxidant activity of einkorn and bread wheat

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Abstract The whole meal flour of wheat is rich in phenolic acids, which provide a relevant antioxidant activity to food products. Aim of this research was to assess the influence of processing on phenolic acid content and antioxidant activity of whole meal flour water biscuits and puffed kernels of einkorn and bread wheat. To this end, the evolution of syringaldehyde, ferulic, vanillic, syringic, *p*-coumaric, *p*-hydroxybenzoic, and caffeic acids was studied during manufacturing. Overall, from flour to water biscuit, the total soluble conjugated phenolic acids increased slightly in einkorn, while the insoluble bound phenolic acids decreased in all the accessions as a consequence of losses during the mixing step. In the puffed kernels, instead, the total soluble conjugated phenolic acids increased markedly, while the

bound phenolics did not show any clear change, evidencing their high thermal stability. The antioxidant activity, measured by FRAP and ABTS, increased during processing and was highest under the most drastic puffing conditions.

Keywords ABTS · Baking · FRAP · Puffing · Wheat

Introduction

Wheat is a staple food for a large part of the world population, with an increasing demand and an estimated consumption of 698 million tons in 2013–2014 (International Grains Council 2014). Mainly valued as a rich source of carbohydrates, wheat provides the daily diet with a relevant proportion of proteins (albeit poor in some essential amino acids such as lysine and tryptophan), fibre, minerals and antioxidant compounds, viz. phenolic acids, carotenoids and tocopherols (Hidalgo and Brandolini 2014; Ward et al. 2008).

Phenolic acids are the most common phenolic compounds in cereals (Li et al. 2008), where are present in three forms: soluble free, soluble conjugated and insoluble bound (Naczek and Shahidi 2004). The soluble conjugated are esterified to sugars and other low molecular weight components, the insoluble bound are esterified to oligo- or polysaccharides and form bridges between polymer chains in the kernel. While the free forms can be extracted using organic solvents, the extraction of bound and conjugated phenolic acids requires acid or base hydrolysis to release them from the cell wall matrix. In wheat, the most abundant fraction is the insoluble bound (77 %), followed by the soluble conjugated (22 %) and the soluble free (<0.5–1 %) (Li et al. 2008). Ferulic acid is the most abundant compound, both in the soluble conjugated and in the insoluble bound forms (Brandolini et al. 2013). The phenolic acids are limited to the outer layers of the wheat

Highlights • Phenolic acids were analysed during water biscuit and puffed kernel processing

- Total phenolic acids decreased during the water biscuit mixing step
- Conjugated phenolic acids increased in all samples during puffing
- Antioxidant activity increased in water biscuits and puffed kernels
- Puffing induced stronger changes than biscuit-making

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kernel (Barron et al. 2007; Brandolini et al. 2013), therefore whole meal flour still has all the antioxidants of the whole grain, while for the production of white flour removes germ and bran, leading to a significant loss of polyphenols (Ragaei et al. 2014).

Water biscuits are popular bakery foods prepared using only flour and water, without the addition of shortening or other fats usually employed in biscuit production; their simple composition makes them perfect for the study of the changes in antioxidants during their production. Puffed kernels are a key component of breakfast cereals and snacks, two steadily increasing food commodities. Puffed kernels are prepared from whole wheat kernels and as such retain most of their nutritional value, with the additional advantage of enhanced stability as a consequence of the inhibition of the enzymes lipase and lipoxygenase (Ragaei et al. 2014). However very few studies are available in literature about puffing and its effect on kernel characteristics, and nothing is known about the changes in phenolic acids content and antioxidant properties.

In fact, food manufacturing modifies antioxidant content, composition and bioavailability. The evolution of carotenoids and tocopherols during the production of bakery products and pasta is a well studied phenomenon; their decrease has been attributed to enzymatic activity (Borrelli et al. 1999; Hidalgo and Brandolini 2010; Hidalgo et al. 2010; Leenhardt et al. 2006) and, for carotenoids, to heat degradation (Hidalgo et al. 2010). Contrasting results are instead published on phenolic acids evolution during food manufacturing (Abdel-Aal and Rabalski 2013; Fares et al. 2010; Gélinas and McKinnon 2006; Menga et al. 2010; Moore et al. 2009). Additionally, food processing often favours Maillard reaction development, leading to a loss of nutritional properties but to an increase of antioxidant activity (Gallegos-Infante et al. 2010).

Increasing attention about the nutritional quality of foods has fostered renewed interest on the hulled wheat einkorn (*Triticum monococcum* L. ssp. *monococcum*). This ancient wheat is rich in proteins, lipids (mostly unsaturated fatty acids), fructans, trace elements (including zinc and iron) and some antioxidant compounds (carotenoids, tocopherols, conjugated polyphenols), as well as shows low β -amylase and lipoxygenase activities, which limit heat damage and antioxidant degradation during food processing, respectively (Hidalgo and Brandolini 2014). The excellent nutritional properties of its flour, superior to those of other wheats, and current trends towards the consumption of functional foods suggest that this cereal may still play a significant role in human consumption, especially in new or special foods with superior nutritional quality.

Aim of this research was therefore to assess the effect of water biscuit processing and kernel puffing of einkorn and bread wheat on phenolic acid content and antioxidant activity.

Materials and methods

Materials Three einkorns (cv. Monlis, Sal98-32 and Monarca) and four bread wheats (cv. Bandera, Masaccio, Blasco and Bramante,) were cropped in 2010–2011 at Sant'Angelo Lodigiano (Po Plain, Lodi, Italy) in 10 m² plots arranged in a randomised complete block design with three replications, following standard cultural practices. After harvesting, the kernels from the three replications were merged and stored at 5 °C. Before processing the seeds of Monlis and Monarca were de-hulled with an Otake FC4S thresher (Satake, Japan); dehulling was not necessary for the other wheats, all free-threshing. Whole meal flour for biscuit preparation was obtained from Monlis, SAL98-32, Bandera and Masaccio kernels ground with a Cyclotec 1093 lab mill (FOSS Tecator, Denmark); kernels of Monlis, Monarca, Blasco and Bramante were used for puffing.

Water biscuits preparation The water biscuits (WB) were manufactured as described by Hidalgo and Brandolini (2011), using only whole meal flour (30 g dry weight basis for each WB) and water, to unambiguously determine the role of flour in phenolic acid changes. Optimum water quantity and mixing time of each sample were determined by Brabender farinograph analysis. Dough mixing was carried out with a National cookie dough micromixer (National Mfg. Co, Lincoln, Nebraska, USA). The dough was transferred to ungreased cookie sheets (covered with parchment paper), rolled to 7 mm thickness using gauge strips and a rolling pin, and cut with a cookie cutter (inside diameter: 60 mm). Baking was carried out at 205 °C for 25 min or for 35 min; four WB were prepared for each accession and each baking time.

Samples of whole meal flour (Flour), just made dough (Dough) and baked water biscuits (25 min and 35 min) were collected and stored at −20 °C until analysis. Deep-frozen mixes and WB were finely ground with a Commercial Heavy-Duty Blender (Waring, Torrington, USA) just before analysis.

Kernel puffing The seeds of each genotype were treated in a gun-puffing system of a private food industry under two different processing conditions:

- Standard. The seeds were fed into a cooker, heated to 380 °C and held at that temperature for 4 min. Puffing took place in an expansion chamber where the precooked grains came into contact for 2.5 min with steam injected under pressure (8.5 bar).
- Optimal. The conditions changed among genotypes to achieve maximum expansion and yield. Cooking was performed for 3–5 min at 360–390 °C while puffing was done under steam pressure between 7 and 10 bar for 1–4 min.

The standard puffing conditions were identical across samples and were designed to allow the comparison of the results without the hindrance of varying puffing conditions; on the other hand, the optimal conditions were specifically determined for each sample, with the aim to achieve the maximal expansion of the kernels. Immediately before analysis the untreated kernels used as controls were ground to whole meal flour with a Cyclotec 1093 lab mill (FOSS Tecator, Denmark), while the puffed kernels (PK) were ground with a Commercial Heavy-Duty Blender (Waring, Torrington, USA).

Chemical analysis Dry matter (DM) content was determined following method 44-15.02 (AACC International). The extraction and analysis by RP-HPLC of the soluble conjugated and insoluble bound phenolic acids were performed as described by Brandolini et al. (2013).

Extraction of soluble conjugated and insoluble bound phenolic acids for HPLC analysis Exactly 0.5 g of whole meal flour were mixed with 15 mL of a methanol/acetone/water (7:7:6) solution. After 15 min in an ice bath under discontinuous vortexing and after centrifugation (11,178g, 10 min, 8 °C) with a Centrikon K24 centrifuge (Kontron Instruments, Bletchley, UK), the supernatant was recovered; the extraction from the sediment was repeated twice, and the three extracts were pooled.

For the determination of soluble conjugated phenolic compounds, the pooled supernatant was evaporated under vacuum at 35 °C for 18 min with a rotator evaporator Laborota 4000 (Heidolph, Milan, Italy), and nitrogen flux for 1 min, while for the insoluble bound phenolic compounds the sediment was analysed. Both the evaporated supernatant and the sediment were digested with 15 mL of 4 M NaOH under nitrogen for 4 h at room temperature and continuous shaking, brought to pH 1.5–2 with 6 M HCl and extracted twice with 20 mL of diethyl ether/ethyl acetate (1:1 v/v). The extracts were then clarified with sodium sulphate, filtered with glass fibre 110 mm (Whatman, Maidstone, England), evaporated as previously outlined, resuspended in 2 mL of methanol:water (1:1 v/v) and filtered with a 0.22 µm PTFE membrane (Millipore, Carrigtwohill Co., Cork, Ireland). All extractions were performed in duplicate under dim light; the extraction tubes were wrapped with aluminium foil, to avoid sample degradation by photo-oxidation.

HPLC determination The following operating conditions were adopted: column Altima C18 5 µm 4.6 mm×250 mm (Grace Davison Discovery Sciences, Deerfield, IL, USA), precolumn Altima C18 5 µm 4.6 mm×10 mm (Grace Davison Discovery Sciences, Deerfield, IL, USA) thermostated at 30 °C; pump L-2130 Elite La Chrom (VWR, Hitachi, Japan), column oven L-2300 Elite La Chrom (VWR, Hitachi, Japan); mobile phase: A) 1 % (v/v) acetic acid in water, B) methanol;

flow rate 1.5 mL/min. The gradient, in terms of eluent B, was: at time 0, 15 %; at 10 min, 20 %; at 16 min, 23 %; at 24–28, 27 %; at 30–34, 15 %. The HPLC system was controlled by the software EZChrom Client/Server version 3.1.7. The compounds were detected at 280 nm with a Diode Array Detector L2450 Elite La Chrom (Merck, Hitachi, Japan). For peak quantification, the calibration curves were constructed using standards from Sigma-Aldrich (St. Louis, MO, USA): caffeic acid (between 0 and 7.29 mg/L), ferulic acid (0–200.38 mg/L), *p*-coumaric acid (0–9.93 mg/L), *p*-hydroxybenzoic acid (0–26.48 mg/L), syringaldehyde (0–11.44 mg/L), syringic acid (0–19.62 mg/L), vanillic acid (0–19.58 mg/L). The calibration curves were linear in the concentration intervals assessed. The results are expressed as mg/kg DM.

Extraction procedure for antioxidant activity assays Exactly 0.5 g of sample (whole meal flour, dough, baked biscuit and/or puffed kernel) were weighed and extracted with 5 mL of methanol:water (80:20 v:v) solution acidified with 1 % HCl (MeOH:HCl) (Fares et al. 2010) under agitation using Vortex (30 s) and a multi-rotator stirrer PTR-35 (Grant-bio, England) for 30 min at 4 °C in the dark. The mixtures were centrifuged at 11,200g per 10 min at 8 °C and the supernatants collected. The residues were re-extracted under the same conditions, resulting in 10 mL crude extract.

Saturated butanol (BuOH) extracts were obtained performing a single extraction with 0.5 g of sample and 10 mL of solvent under agitation with Vortex (30 s) and orbital stirrer (2 h) at 4 °C in the dark followed by centrifugation as formerly described.

ABTS antioxidant activity assay The ABTS radical scavenging capacity was analysed as described by Yilmaz et al. (2015). A stable stock solution of the ABTS radical cation was prepared by reacting 10 mL of an aqueous solution of 2,2'-azino-bis-3-ethylenbenzothiazoline 7 mM and 176 µL potassium persulfate 140 mM in the dark at room temperature for 12–16 h before use. Saturated butanol extracts (150 µL) reacted with 5 mL of a diluted ABTS radical solution in ethanol (absorbance: 0.70 ± 0.02 AU at 734 nm); the absorbance was measured after 6 min at 30 °C at a wavelength of 734 nm with a spectrophotometer model V650 (Jasco, Japan), using ethanol as blank. Antioxidant activity was evaluated as percentage of absorbance decrease (inhibition percentage). A reference curve was built with 11 concentrations (from 0.05 to 0.72 mM) of Trolox (Sigma-Aldrich St. Louis, MO, USA). The results were expressed as mmol of Trolox equivalents (TE)/kg DM.

FRAP antioxidant activity assay The ferric reducing antioxidant power (FRAP) was determined as described by Yilmaz et al. (2015). Briefly, 200 µL of saturated butanol or MeOH:HCl extracts were mixed with 4.5 mL of FRAP reagent. Absorption was measured after 60 min incubation at 37 °C with

a spectrophotometer (model V650, Jasco, Japan) at a wavelength of 593 nm; acetate buffer 0.3 M pH 3.6 was used as blank. The FRAP reagent, prepared daily, consisted of 0.3 M acetate buffer (pH 3.6), 10 mM 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl and 20 mM FeCl₃ (ratio: 10:1:1 v/v/v). FRAP values were obtained by comparing the results to a calibration curve built with 18 concentrations (0.06–0.90 mM) of Trolox (Sigma-Aldrich, St. Louis, MO, USA). The antioxidant activity was expressed as mmol TE/kg DM.

Statistical analysis For each product (WB and PK) a two-way ANOVA considering genotype and processing step as factors was performed; a separate one-way ANOVA was carried out on Monlis final products to compare the effect of the different treatments. Afterwards Fisher's least significant difference (LSD) test at $p \leq 0.05$ and Pearson's correlations of the means were performed. The analyses were executed using the software STATGRAPHICS Centurion XVI for Windows (StatPoint Technologies, Inc. Warrenton, VA, USA).

Results and discussion

Processing effect on phenolic acids Figures 1 and 2 describe the evolution of soluble conjugated phenolic acids, and Figs. 3 and 4 the evolution of insoluble bound phenolic acids in WB and PK, respectively. The conjugated phenolic acids identified were ferulic, vanillic, syringic, *p*-coumaric, *p*-hydroxybenzoic and syringaldehyde. The bound phenolic acids were ferulic, *p*-coumaric, caffeic, vanillic, syringic, and *p*-hydroxybenzoic. The einkorn whole meal flour samples were richer in total conjugated acids (56.6 vs. 44.5 mg/kg DM) and poorer in total bound phenolic acids (528.3 vs. 616.1 mg/kg DM) than bread wheat; ferulic acid was always the most abundant compound, representing 53–73 % of total conjugated and 91–95 % of total bound phenolic acids. These values fit exactly with the results reported by Brandolini et al. (2013) for their analysis of 39 different *Triticum* spp. accessions and are very similar to the data by Moore et al. (2005), Okarter et al. (2010), and Serpen et al. (2008).

The ANOVA (Supplementary Tables 1 and 2), carried out separately for WB and PK, indicated that the processing steps had significant influence, except for conjugated ferulic acid in WB and for bound ferulic acid in PK. The genotype effect was always significant. The processing step (or treatment) $\times$ genotype interaction was significant for the conjugated phenolic acids *p*-hydroxybenzoic, vanillic and syringaldehyde in WB, and for *p*-hydroxybenzoic, vanillic, syringic and *p*-coumaric, in PK. Similarly the interaction was significant for the bound phenolic acids vanillic, caffeic, ferulic and total in WB, and *p*-hydroxybenzoic, vanillic, syringic, caffeic and *p*-coumaric in PK. All the significant interactions were however of minor importance compared to the main effects.

During the WB mixing step (Fig. 1), a decrease was detected only for soluble conjugated *p*-hydroxybenzoic acid (on average 23 %), syringic acid (12 %) and syringaldehyde (20 %). Significant reductions of all the compounds were instead recorded for bound phenolic acids (Fig. 3), possibly because of enzymatic activity (Hidalgo et al. 2013); the average losses were 13 % for ferulic acid, 17 % for *p*-coumaric acid, 32 % for caffeic acid, 9 % for vanillic acid, 17 % for syringic acid, and 35 % for *p*-hydroxybenzoic acid. These results are consistent with the observations of Moore et al. (2009), which reported that fermentation significantly decreased either soluble conjugated or insoluble bound ferulic acid, and of Boskov Hansen et al. (2002), which observed an 8.6 % reduction of the total bound phenolic acids, due to ferulic acid decrease, during the preparation of rye bread acid dough. While manufacturing durum wheat pasta, Fares et al. (2010) also noticed a decrease in free phenolic acids, but did not record changes in bound phenolic acids content.

During baking conjugated vanillic acid, syringic acid and syringaldehyde increased in all samples, but the total conjugated acids augmented only in einkorn and after 35 min (Fig. 1). The bound *p*-hydroxybenzoic, caffeic and vanillic acids generally increased, and the total bound phenolic acids increased slightly in three out of four samples (Fig. 3). Differently, Abdel-Aal and Rabalski (2013) observed a reduction of bound phenolics and an increase of free phenolics during the baking of einkorn whole meal bread, biscuits and muffins. On the other hand, Menga et al. (2010) observed a reduction in free (22.5 %) as well as in bound phenolics (6–10 %) in bran-enriched bread baked at 220 °C for 30 min, Moore et al. (2009) reported a drop in conjugated and no changes in bound ferulic acid in pizza crust after baking, and Boskov Hansen et al. (2002) observed a small decline in ester-bound phenolic acids during rye bread baking at 200 °C for 35 min. At lower temperatures, during pasta cooking, Fares et al. (2010) observed a limited loss of free phenolic acids but a consistent increase in bound phenolic acids.

Overall, from flour to WB, the soluble conjugated phenolic acids increased slightly only in the einkorn WB baked for 35 min, while the insoluble bound phenolic acids always decreased in all the accessions, as a consequence of losses during the mixing step. Gélinas and McKinnon (2006) described a slight increase of total phenolic compounds in whole meal bread compared to starting whole meal; additionally, they observed that the crust of white bread contained more phenolic compounds than the crumb, a difference not observed in the whole meal product.

Concerning the PK, almost all the conjugated compounds, excluding *p*-hydroxybenzoic and *p*-coumaric acids, showed a remarkable raise after puffing (Fig. 2), leading to an overall total conjugated phenolic acid increase of around 70 %, far higher than that observed during WB baking. This increase could be probably related to more drastic thermal conditions

Fig. 1 Content in soluble conjugated phenolic acids from whole meal flour, dough and water biscuits baked at 205 °C for 25 and 35 min of two einkorns (Monlis, Sal 98-32) and two bread wheats (Bandera, Masaccio); the error bars represent the standard error

involved by puffing than by biscuit-making. In fact, puffing led to stronger heat damage than baking (Cattaneo et al. 2015; Hidalgo and Brandolini 2011). The optimal puffing treatment, which induced the strongest heat damage (Cattaneo et al. 2015), in almost all cases determined the highest concentration rise. The bound fraction of PK, instead, did not change significantly (Fig. 4), behaviour similar to that observed during WB baking. Thus, the higher amount of several soluble

conjugated phenolic acids after thermal treatment does not seem to originate from the insoluble bound fraction, but might be related to a heat-induced rupture of some ester bonds, leading to an increase of extractable compounds. Direct comparisons with published data were impossible because, to the best of our knowledge, no literature on phenolic acids in puffed cereals is available. However, whole-meal flour of wheat, barley, rye and oat extruded at temperatures ranging from 160 to

Fig. 2 Content in soluble conjugated phenolic acids from raw and puffed kernels (optimal and standard conditions) of two einkorns (Monlis and Monarca) and two bread wheats (Blasco and Bramante); the *error bars* represent the standard error

200 °C showed an increase in free and bound phenolic acids, possibly because the hydrothermal treatment liberated bound phenolic acids from cell walls (Zielinski et al. 2001).

The einkorn cv Monlis, which was used for preparing both water biscuits and puffed kernels, allowed a more direct comparison of the changes in soluble conjugated and insoluble bound concentration observed with the two processing treatments. The soluble conjugated vanillic, syringic and ferulic

acids were higher in the PK than in the WB; overall, the PK had about 57 % more soluble conjugated phenolic acids than the WB. Among insoluble bound phenolics, *p*-hydroxybenzoic and ferulic acid showed a moderately superior content in PK than in WB, while caffeic acid was inferior; overall, the total bound phenolics were more abundant by 10 %. On the whole, the change in total phenolic acids observed in PK vs. WB was 15 %.

Fig. 3 Content in insoluble bound phenolic acids from whole meal flour, dough and water biscuits baked at 205 °C for 25 and 35 min of two einkorns (Monlis, Sal 98-32) and two bread wheats (Bandera, Masaccio); the error bars represent the standard error

Processing effect on antioxidant activity The antioxidant activity during WB preparation changed significantly. When assessed on saturated butanol extracts (Fig. 5a) by the ABTS test, a slight decrease during the mixing step followed by a small increase was detected for einkorn after baking for 25 min, but not for bread wheat; only Monlis showed further growth after baking for 35 min. On the other hand, when the antioxidant activity was evaluated by the FRAP test on butanol

(Fig. 5b) or on acidified methanol extracts (Fig. 5c) the small decline from flour to mix was followed by an increase after baking, which was usually stronger after 35 min (37.1 % and 78.1 % for $FRAP_{BuOH}$ and $FRAP_{MeOH:HCl}$ respectively) than after 25 min (21.5 % and 36.1 %). After puffing, all the three tests ($ABTS_{BuOH}$, $FRAP_{BuOH}$ and $FRAP_{MeOH:HCl}$) showed a strong boost in the antioxidant activity of the samples compared to the raw kernels (Fig. 5d, e and f), with 106 %, 214 % and

Fig. 4 Content in insoluble bound phenolic acids from raw kernels and puffed kernels (optimal and standard conditions) of two einkorns (Monlis and Monarca) and two bread wheats (Blasco and Bramante); the error bars represent the standard error

378 % increases for the standard treatment, and 113 %, 231 % and 402 % for the optimised treatment. Comparing the two processes, it is clear that the antioxidant activity of PK was far higher than that of WB. In fact, comparing the results of the einkorn cv. Monlis, which was used for both products (Fig. 5), ABTS_{BuOH} showed a 122 %, FRAP_{BuOH} a 199 % and FRAP_{MeOH:HCl} a 159 % difference.

The antioxidant activity increase during processing does not seem totally justified by the changes in

phenolic acid concentration, nor in other antioxidant compounds such as carotenoids and tocopherols, which diminish during processing (Hidalgo and Brandolini 2010; Hidalgo et al. 2010). Several authors reported an increase of antioxidant activity in biscuits as a consequence of heat damage (Haase et al. 2012; Morales et al. 2009; Virág et al. 2013). Similarly, Moore et al. (2009) observed a raise of antioxidant activity in whole-wheat pizza crust after baking, and the effect

Fig. 5 Antioxidant activity, measured by ABTS and FRAP tests, of Monlis water biscuits and puffed kernels extracts in saturated butanol (BuOH) and/or methanol acidified with hydrochloric acid (MeOH:HCl)

was stronger with longer cooking time or higher temperature, Capuano et al. (2009) showed that the increase in antioxidant activity of bread crisps in function of toasting time was correlated to HMF and acrylamide, and Gallegos-Infante et al. (2010), studying the effect of traditional Mexican processing of barley, reported higher antioxidant activity of the roasted or cooked germinated kernels than of the control. An increase in antioxidant activity for barley grits extruded at temperatures varying from 150 to 180 °C was observed by Sharma et al. (2012), and tentatively associated with some dark colour pigments (particularly melanoidins) generated during the heat processing of foods. In fact, Osada and Shibamoto (2006) observed a high antioxidant activity in volatile extracts obtained from non-enzymatic browning model systems because of the combined and synergistic effect of many compounds produced by the Maillard reaction.

Correlations The analysis of the correlation (Supplementary Tables 3 and 4) showed that for both WB ($n=16$) and PK ($n=12$) the antioxidant activity was largely linked to the conjugated phenolic acids. In the case of WB, the correlation was 0.88, 0.83 and 0.67 ($p \leq 0.001$) for ABTS_{BuOH}, FRAP_{BuOH} and FRAP_{MeOH:HCl} respectively, while for PK it was 0.94, 0.93 and 0.92 ($p \leq 0.001$). The conjugated phenolic acids most correlated to the antioxidant activity were (in decreasing order) *p*-coumaric, ferulic and vanillic for WB, and vanillic, ferulic and *p*-coumaric for PK. The correlations between bound phenolic acids and antioxidant activity, instead, were generally not significant. A positive correlation between total soluble phenolic compounds and antioxidant activity was noticed by Pasqualone et al. (2014) analysing a collection of 113 tetraploid wheat samples. Similarly, Adom et al. (2003), analysing 11 wheat varieties, reported a significant positive correlation between total ferulic or total phenolic content (free+bound) and antioxidant activity.

Conclusions

Phenolic acids were quite stable during the preparation of water biscuits and puffed kernels. In fact, after a small decrease during the mixing step (for water biscuits), the soluble conjugated phenolic acids increased during baking in einkorn, and during puffing in all accessions, while the insoluble bound did not change. More extreme thermal conditions, such as those applied during puffing, fostered a stronger augment. The antioxidant activity of water biscuits and puffed kernels was consistently greater than that of the raw materials.

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