Microencapsulates and extracts from red beetroot pomace modify antioxidant capacity, heat damage and colour of pseudocereals-enriched einkorn water biscuits

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Abstract
Cereals supply humankind with carbohydrates, proteins and several health-enhancing compounds, including antioxidants. Pomace, a by-product of beetroot juice preparation, is rich in antioxidants (phenolic compounds and betalains). The aim of this work was to study the effect of pomace extract addition, either pure or microencapsulated, on antioxidant properties, heat damage and colour of einkorn water biscuits enriched with pseudocereals. Pomace extract addition had different effects on total polyphenol contents and antioxidant capacity (FRAP and ABTS) in diverse blends. In bread, wheat and einkorn matrices, a significant increase was observed, while in pseudocereals-enriched blends, richer in antioxidants, only microencapsulation improved their content. Pomace extract addition led to furosine reduction and hydroxymethylfurfural increase. Microencapsulate-enriched WB were richer in betanin, isobetanin, total phenolics and antioxidant capacity. In conclusion, pomace extracts, by-products of juice manufacturing, significantly improve some nutritional characteristics of baked products, especially when conveyed as microencapsulates.

Keywords: ABTS; Amaranth; Betacyanins; Buckwheat; FRAP; Quinoa

1 Introduction
Cereals are the staple food of humankind and, besides supplying most of our daily energy requirement, provide numerous compounds with health-enhancing properties. The high consumption of cereal-based products implies that even small variations in the concentration of such compounds may have a positive effect on human health. Due to the high carbohydrate contents of bakery foods, several studies suggest the partial replacement of refined wheat flour with other ingredients, rich in bioactive compounds, to improve their nutritional composition (de Camargo, Vidal, Canniatti-Brazaca, & Shahidi, 2014).

Some underutilised crops, such as einkorn (Triticum monococcum L. ssp. monococcum) and the pseudocereals buckwheat (Fagopyrum esculentum), quinoa (Chenopodium quinoa) and amaranth (Amaranthus spp.), contain relevant amounts of nutritionally valuable molecules, notably antioxidant compounds, such as phenolic acids, polyphenols, carotenoids and tocots (Alvarez-Jubete, Wijngaard, Arendt, & Gallagher, 2010; Hidalgo & Brandolini, 2014). In particular phenolic acids, the most common type of phenolic compounds in cereals (Li, Shewry, & Ward, 2008), are present in three forms: soluble free, soluble conjugated, i.e. esterified to sugars and other low molecular weight components, and insoluble bound, i.e. linked to cell wall constituents such as polysaccharides, protein, lignin, cutin or suberin (Naczk & Shahidi, 2004). In wheat, insoluble bound is the most abundant fraction (77%), followed by soluble conjugated (22%) and soluble free (<0.5–1%) (Li et al., 2008). Bound phenolic acids are highly stable under heat treatments (Hidalgo, Yilmaz, & Brandolini, 2016) but have poor nutritional significance because of low bioaccessibility; the scarce free form, instead, is the most bioavailable and the least stable. Therefore, adding ingredients rich in free phenolics could improve the polyphenols composition of foods.
The red beetroot (Beta vulgaris L.), largely used for the preparation of fresh and canned foods, contains significant amount of phenolic acids, such as ferulic, protocatechuic, vanillic, p-coumaric, p-hydroxybenzoic and syringic acids (Kujala, Loponen, Kikka, & Pihlaja, 2000). Besides polyphenols, beetroot contains betalains, plant pigments which couple their strong colouring properties with high antioxidant capacity (Ravichandran, Ahmed, Knorr, & Smetanska, 2012). Betanin (betanidin 5-D-glucoside; CI Natural Red 33; E-number E162), the main pigment in red beet, is the only betalain approved for use in food (Pires Gonçalves et al., 2012). Betanin is concentrated in the red beetroot peel; therefore its functions could be related to plant defence mechanisms, such as photoprotection, increased pathogen resistance, and antioxidant activities (Sintzing & Carle, 2004). Interestingly, the biologically beneficial properties of betalains are also maintained after ingestion. For example, Allegra et al. (2015) showed that indicaxanthin, after crossing the intestinal epithelial cell monolayer, was absorbed through paracellular junctions, was found in human plasma at a peak concentration 3 h after the ingestion and exhibited an anti-inflammatory effect in a carrageenan-induced acute inflammation model.

In several central and eastern European countries red beetroot is widely utilised for the production of juice, for direct consumption or as a food colorant (Janiszewska, 2014). The exhausted red beetroot pulp (pomace), a by-product of juice manufacturing, still contains significant concentrations of phenolic compounds and betalains (Vučić et al., 2012, 2014).

Many bioactive ingredients are characterised by chemical instability, and are prone to destruction during food processing and storage (Hidalgo & Brandolini, 2010; Hidalgo, Brandolini, & Pompei, 2010). Above 50 °C betalains are very sensitive to degradation, a major drawback for their use as food colorants. Herbach, Sintzing, and Carle (2006) report that betacyanins degrade upon exposure to higher temperatures, forming yellow products, such as betalamic acid, neobetacyanins, and betaxanthins; furthermore, decarboxylation reactions and removal of the glycoside unit are described (Kaimainen, 2014).

Nevertheless, the degradation of bioactive compounds is often reduced by microencapsulation (Dias, Ferreira, & Barreiro, 2015). Additionally, microencapsulated pigments are easier to handle, have better solubility, stability, flow properties and reduce dusting when added to dry mixtures (Gibbs, Kermasha, Ali, & Mulligan, 1999). Among microencapsulation techniques, spray-drying is the most extensively used, while freeze drying, although more expensive, has the advantage that no heating is applied (Lopez-Quiroga, Antelo, & Alonso, 2012). In recent years microencapsulation techniques have been widely studied, but the behaviour of microencapsulated compounds in formulations and foods remains largely unknown (Dias et al., 2015).

The aim of this work was thus to study the effect of the addition of red beetroot pomace extracts, either pure or microencapsulated, on antioxidant properties, thermal damage and colour of einkorn water biscuits enriched with pseudocereals.

2 Materials and methods

2.1 Samples

2.1.1 Flours/blends

The flours were obtained from einkorn wheat (Triticum monococcum L. sp. monococcum cv. Monlis), bread wheat (Triticum aestivum L. sp. aestivum cv. Bramante), amaranth (Amaranthus cruentus L. cv. MT-3), buckwheat (Polygonum fagopyrum Moench local population Seis) and quinoa (Chenopodium quinoa Willd.). Einkorn wheat, bread wheat, amaranth and buckwheat were produced in 2014 in the fields of the Council for research in agriculture and agricultural economy analysis (CREA) in Sant’Angelo Lodigiano (LO), while quinoa was retrieved from the commercial circuit. After harvesting the seeds were stored at 5 °C. Immediately before milling the hulled kernels (Monlis) were dehulled with an Otake FC4S threshing (Satake, Japan). The refined flours of the two wheats were obtained with a lab mill (Bona, Italy), which separates flour from germ and bran; the wholemeal flours of the three pseudocereals were prepared with a Cyclotec 1093 lab mill (FOSS Tecator, Denmark).

2.1.2 Extract preparation

The red beetroot (Beta vulgaris L., cv. ‘Bicor’) for extracts and microencapsulate preparation was purchased at a local supermarket. Beetroots were washed, cut and blended with a laboratory blender (Neo SK-400, TCL King Electrical Appliances Co. Ltd., China). The pomace was separated from the juice by vacuum filtration. The wet pomace was freeze-dried (1–4 LSC model, Martin Christ, Germany). The dry pomace underwent extraction with an ethanol:0.5% acetic acid (83.3:16.7) solution. After 30 min of ultrasound in a water bath at 24–25 °C, the sample was centrifuged for 10 min at 9000 rpm, using an RC 5B Plus centrifuge (Sorvall, USA). To eliminate any residual pulp, a vacuum filtration, using Whatman paper n° 4 and a vacuum pump (KNF Laboport, USA) was performed. Extract concentration was carried out at 35 °C under vacuum by a Rotavapor (Borbon Efficient 4000, Heidelberg, Germany) until reaching 11.51 g dry matter (DM)/100 g (Ex 1) or 26.28 g DM/100 g (Ex 2). The extract for microencapsulate preparation instead was concentrated to 6.87 g DM/100 g.

2.1.3 Microencapsulate preparation

The microencapsulation was obtained by freeze-drying (1–4 LSC model, Martin Christ, Germany), using soy protein isolate as carrier. Briefly: 900 ml of pomace with 6.87 g DM/100 g were mixed with 45 g of soy protein isolate retrieved from the commercial circuit (Soja Protein Macrobiotic Prom, Serbia), having 90% minimum protein content and 5.5% moisture; the mixture was then lyophilised for 48 h.

2.1.4 Water biscuit preparation

To avoid interferences by other ingredients (lipids, sugar and milk powder), normally used in cookie formulations, water biscuits (WB) were produced using only either deionised water and flour or deionised water, flour, extract or microencapsulate. For the preparation of control WB, 80 g of flour at 14% moisture and 36 ml of water were mixed for 90 s, using a Hobart C-100 electric mixer (National MFG CO, Lincoln, Nebraska, U.S.A.). For the preparation of pomace extract (PE) – enriched WB, deionised water was replaced with an equal amount of Ex 1 extract plus 4 ml of water (PE 5.7% DM), or 80 g of flour with 30.5 ml of Ex 2 extract plus 12 ml of water (PE 10.4% DM) or 80 g of flour with 45.7 ml of Ex 2 extract plus 5 ml of water (PE 14.9% DM). Finally, microencapsulate-enriched WB (PME 10.8% DM) were obtained from 68 g of flour, 12 g of microencapsulate and 4 ml of water.
36 ml deionised water. The dough was rolled to obtain a homogeneous 3.9 mm high sheet and cut with a die cutter (inner diameter 35 mm), giving sixteen dough disks of the same size. The disks were immediately baked in an Ovenlab rotary oven (MFG CO National, Lincoln, Nebraska, U.S.A.) at 205 °C for 11.5 min, cooled at room temperature for 30 min and stored at -20 °C.

Different types of WB were prepared, employing five different flour blends: 100% refined einkorn flour (E), 70% einkorn – 30% amaranth (A 30%), 70% einkorn – 35% quinoa (Q 35%), 70% einkorn – 30% buckwheat (BU 30%), 100% refined bread wheat flour (BW); the WB incorporating pomace extracts or microencapsulate were prepared similarly, starting from the five flours/blends. The WB were stored at -20 °C; before analysis, they were ground with a lab mill (Braun, Germany).

2.2 Analyses

2.2.1 General

The flours were characterised for dry matter (method 44-15A, AACC, 1995), ash (method 08-03, AACC, 1995), protein (N × 5.7, Kjeldal test, method 46–10, AACC, 1995), sugar and furosine as described by Hidalgo and Brandolini (2011). The following analyses were performed on WB, extracts and microencapsulates: dry matter (see above), protein content (see above), furosine (see above) and HMF (Rufán-Henares, Delgado-Andrade, & Morales, 2006).

2.2.2 Betacyanins

Betacyanins were extracted and analysed as follows: exactly 0.3 g of ground WB, or 0.2 g of microencapsulate, were put into 2 ml Eppendorf vials. Four extraction cycles were carried out for the WB enriched with pomace extracts, and seven for those enriched with microencapsulate. Individual extractions were made by adding 1.5, 1.5, 1.0, 1.5, 1.0 and 1.0 ml of methanol:H2O:acetic acid (50:42:8). The samples were mixed by Vortex (Reax 2000, Meindolph Heidolph, Germany) for 1 min, ultrasonicated at 8 °C (FS200b, Decon, UK) for 20 min, and orbital agitator (Multi-Rotator GRANT-BIO, UK) for 20 min. After each extraction, the samples were centrifuged at 11,200 g for 5 min at 8 °C (4224 Centrifuge, ALC, Italy) and the supernatants collected in a single test tube. All operations were carried out under dark conditions, to avoid oxidative phenomena.

The extracts were filtered with 0.2 μm PVDF filters (Supelco, USA). Betacyanin quantification was done by HPLC according to the method of Gandía-Herrero, Simón-Carrillo, Escribano, and García-Carmona (2012). A volume of 20 μl of filtrate was analysed under the following operating conditions: Prevail C18 column 5 μm 250 × 4.6 mm (Alltech Italia, Italy) with Prevail C18 pre-column 5 μm 75 × 4.6 mm (Alltech Italia, Italy); 25 °C oven for column L-2300 Elite LaChrom (Hitachi, Japan); pump L-2130 Elite LaChrom (Hitachi, Japan). Mobile phase: solvent A, consisting of trifluoroacetic acid (TFA)-water 0.05% (v/v); solvent B was TFA-acetonitrile 0.05% (v/v) at an operating flow of 1 ml/min. The linear gradient increased in 15 min from 0% B to 35% (v/v) B; the solvent B was maintained at 35% for 5 min, and then returned to starting conditions (0%) for an additional 5 min (total run time 25 min). The compounds were detected at 477 and 538 nm by Diode Array Detector L2450 Elite LaChrom (Hitachi, Japan) set at wavelengths between 200 and 600 nm. The system was managed by EZChrom Client/Server software version 3.1.7.

For the quantification of betacyanin, the red beet extract CDS000584 (Sigma-Aldrich, Italy) was used. The concentration (c) of the mother-solution was verified by spectrophotometric analysis (c = A/A0; A: absorbance at 538 nm), considering the betanin coefficient (ε) (PM = 550.473 g/mol) in water equal to 60,000 l mol-1 cm-1, as reported by Kugler, Stritzing, and Carle (2004). The calibration curve considered the sum of the areas of the two peaks detected at 538 nm in the standard (betanin retention time 11.2 min, 54% and isobetanin, retention time 11.9 min, 46%). The regression line was linear (r² = 1, p ≤ 0.001) in the concentration range 0–27 mg/l, and had a detection limit of 0.56 mg/l. The analyses were carried out in duplicate and the results expressed as mg/kg DM.

2.2.3 Total polyphenols and antioxidant capacity

Total polyphenols content (TPC) and antioxidant capacity were scored on the extracts obtained, following the same procedure as outlined above for betacyanin analysis. TPC was assessed spectrophotometrically by the Folin–Ciocalteu method, as described by Yilmaz, Brandolini, and Hidalgo (2015). The TPC values of microencapsulate-enriched samples were corrected considering the TPC concentration (2633 mg GAE/kg DM) in the soy protein carrier. Antioxidant capacity was measured by the ferric reducing antioxidant power (FRAP) and 2,2′-azino-bis 3-ethylbenzothiazoline-6-sulphonic acid radical cation scavenging capacity (ABTS)+, as described by Yilmaz et al. (2015).

2.2.4 Colour

Colour was assayed on flours/blends and WB. The coordinates L* (luminosity), a* (red-green), b* (yellow-blue) were determined by a Chroma meter II tristimulus colorimeter (Minolta Italia SpA, Milan, Italy) with high sensitivity silicon photo cell filtered to match CIE (Commission Internationale de l'Eclairage) standard observer response, using the standard-white reflector plate and illuminant C.

All chemical analyses were performed twice and colour evaluations three times.

2.3 Statistical analysis

The results were processed by one-way analysis of variance (ANOVA); when significant differences were detected, Fisher’s least significant difference (LSD) at p ≤ 0.05 was computed. All the analyses were performed using the Statistical Program STATGRAPHICS® plus version 4 (Statpoint Technologies, Inc., Warrenton, VA, USA). The average values, standard error and coefficient of variation were calculated using Excel 2007 (Microsoft, USA).

3 Results and discussion

3.1 Flours, extracts and microencapsulate composition
The ANOVAs (not shown), carried out separately on the flours/blends and on the pomace extracts/microencapsulate, always showed significant differences among samples for all the traits assessed. Table 1 lists the main composition characteristics of wheats and pseudocereals. Unsurprisingly, the wholemeal flours of pseudocereals had significantly higher ash contents than had the refined flours of the two wheats. Nevertheless, protein content was higher in einkorn (13.6 g/100 g DM) than in amaranth, quinoa, buckwheat (13.0, 12.4 and 11.3 g/100 g DM, respectively) and bread wheat (9.31 g/100 g DM). These results are all within the ranges observed by Hidalgo and Brandolini (2008) for bread wheat and einkorn, Wright, Pike, Fairbanks, and Huber (2002) for quinoa, Gross et al. (1989) for amaranth and Hager, Wolter, Jacob, Zannini, and Arendt (2012) for buckwheat. The reducing sugars (fructose, glucose and maltose) were present in small amounts (in total, between 0.14 and 0.18 g/100 g DM), except in the case of quinoa (0.26 g/100 g DM), which showed a relatively high glucose content (0.16 g/100 g DM). Sucrose was abundant in the three pseudocereals (from 1.39 to 2.20 g/100 g DM), but more scarce in common wheat (0.38 g/100 g DM) and einkorn (0.82 g/100 g DM).

Furosine, a heat damage index, was maximum in quinoa (41.4 mg/g100 g protein), more modest in buckwheat (12.5 mg/g100 g protein), bread wheat (9.0 mg/g100 g protein) and einkorn (7.81 mg/g100 g protein), and absent in amaranth. A possible explanation is that quinoa seeds are naturally rich in saponins, toxic substances which are removed before milling by washing or abrasion. Washing must be followed by drying, while mechanical abrasion generates heat; therefore, both treatments may induce the formation of furosine. Concerning the other samples, the milling process needed to obtain meals or flours also generates heat, which causes a limited increase in furosine content. Amaranth, perhaps because its seeds are small and soft, requires only a very rapid milling, therefore preventing furosine formation.

The microencapsulate had a much higher protein content than had the pomace extracts (Table 2), because its carrier material was soy protein isolate. The relatively high furosine content was also due to the same cause, as the process to obtain soy protein isolates includes thermal treatments. Nevertheless, heat damage was not particularly intense since HMF, an indicator of intermediate stages of the Maillard reaction or of caramelization, was below the detection limit.

### Table 1 Ash (g/100 g DM), protein (g/100 g DM), sugars (g/100 g DM) and furosine (mg/g 100 protein) in flour of bread wheat and einkorn, and in wholemeal flour of pseudocereals.

<table>
<thead>
<tr>
<th></th>
<th>Bread wheat</th>
<th>Einkorn</th>
<th>Amaranth</th>
<th>Buckwheat</th>
<th>Quinoa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash</td>
<td>0.66±0.000</td>
<td>0.78±0.015</td>
<td>3.26±0.037</td>
<td>4.35±0.028</td>
<td>2.57±0.049</td>
</tr>
<tr>
<td>Protein</td>
<td>9.31±0.020</td>
<td>13.6±0.050</td>
<td>13.0±0.054</td>
<td>11.3±0.024</td>
<td>12.4±0.058</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.04±0.002</td>
<td>0.04±0.000</td>
<td>0.06±0.002</td>
<td>0.07±0.004</td>
<td>0.06±0.001</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.04±0.000</td>
<td>0.04±0.002</td>
<td>0.04±0.003</td>
<td>0.06±0.001</td>
<td>0.16±0.004</td>
</tr>
<tr>
<td>Maltose</td>
<td>0.07±0.001</td>
<td>0.08±0.003</td>
<td>0.04±0.003</td>
<td>0.05±0.002</td>
<td>0.04±0.001</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.38±0.004</td>
<td>0.82±0.020</td>
<td>1.56±0.061</td>
<td>1.39±0.094</td>
<td>2.20±0.018</td>
</tr>
<tr>
<td>Furosine</td>
<td>9.00±0.251</td>
<td>7.81±0.134</td>
<td>nd</td>
<td>12.5±0.385</td>
<td>41.4±2.085</td>
</tr>
</tbody>
</table>

nd: not detected. Different letters indicate significant differences among samples along rows following the LDS test (p ≤ 0.05).

### Table 2 Contents of protein, betanin, isobetanin, total polyphenols (TPC), furosine, and hydroxymethylfurfural (HMF), and antioxidant capacity (FRAP, ABTS) of the extracts (Ex1 and Ex 2) and of the microincapsulate (PME) from exhausted red beetroot pulps.

<table>
<thead>
<tr>
<th></th>
<th>Ex 1</th>
<th>Ex 2</th>
<th>PME</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (g/100 g)</td>
<td>1.1±0.01</td>
<td>2.3±0.03</td>
<td>33.5±1.50</td>
</tr>
<tr>
<td>Betanin (mg/kg DM)</td>
<td>457±4.62</td>
<td>789±0.27</td>
<td>1377±65.8</td>
</tr>
<tr>
<td>Isobetanin (mg/kg DM)</td>
<td>91.0±1.90</td>
<td>236±1.54</td>
<td>301±24.4</td>
</tr>
<tr>
<td>TPC (mg GAE/kg DM)</td>
<td>1945±11.7</td>
<td>3698±10.7</td>
<td>3791±28.8</td>
</tr>
<tr>
<td>FRAP (mmol TE/kg DM)</td>
<td>9.4±0.51</td>
<td>27.5±1.26</td>
<td>27.7±0.29</td>
</tr>
<tr>
<td>ABTS (mmol TE/kg DM)</td>
<td>13.8±0.00</td>
<td>20.3±0.38</td>
<td>21.5±0.41</td>
</tr>
<tr>
<td>Furosine (mg/g protein)</td>
<td>nd</td>
<td>nd</td>
<td>32.9±0.73</td>
</tr>
<tr>
<td>HMF (mg/100 g)</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

Gross et al. (1989) for amaranth and Hager, Wolter, Jacob, Zannini, and Arendt (2012) for buckwheat. The reducing sugars (fructose, glucose and maltose) were present in small amounts (in total, between 0.14 and 0.18 g/100 g DM), except in the case of quinoa (0.26 g/100 g DM), which showed a relatively high glucose content (0.16 g/100 g DM). Sucrose was abundant in the three pseudocereals (from 1.39 to 2.20 g/100 g DM), but more scarce in common wheat (0.38 g/100 g DM) and einkorn (0.82 g/100 g DM).
Betanin, isobetanin and TPC were significantly more abundant in the microencapsulate than in the Ex2 and Ex1 extracts (Table 2). The betanin and isobetanin values of the more concentrated extract (Ex 2) were higher than those observed by Vučić et al. (2012 and 2014) in pomace of different beetroot varieties and higher than those of betacyanins observed by Janiszewska (2014) and Wruss et al. (2015) in juice (430 mg/kg DM and 465–807 mg/l, respectively). Our TPC results for the concentrated extract were superior to those found by other authors in dried root (Gokhale & Lele, 2014) or fresh pulp (Sreeramulu & Raghunath, 2010), but fitted perfectly with the variation observed in pomace by Vučić et al. (2012).

Overall, these results suggest that microencapsulation shelters antioxidant substances against degradation. Interestingly, microencapsulation also hinders antioxidant extraction for analysis: despite repeated extractions, the microencapsulate sediment was never colourless, thus implying that the results were always underestimated.

Comparing our data with literature results, the antioxidant capacity of the extract was lower than the values reported by Sawicki, Bączek, and Wiczkowski (2016) for whole beetroot (ABTS) but was within the range of variation found by Wruss et al. (2015) for juice (FRAP). However, our extracts were from pomace, where the water-soluble polyphenols were removed during juice extraction, therefore reducing the overall antioxidant capacity.

3.2 Water biscuits

3.2.1 General

The ANOVA (not shown) indicated that pomace concentration was the factor that most influenced betacyanin contents, as well as L* and a* colour coordinates, while type of flour/blend also had a significant effect. On the other hand, type of flour/blend strongly influenced TPC content, antioxidant capacity, furosine and HMF, along with the b* colour coordinate; pomace concentration also exerted a significant influence. The interaction of these factors, although always significant, was of minor importance.

3.2.2 Betacyanins

Betanin and its epimer isobetanin were the only betacyanins detected in the standard and in the extracts, including the microencapsulate. In WB two more compounds were observed, having betanin spectra and retention times of 12.5 and 13.3 min (Fig. 1), and probably were derived from thermal degradation of betanin and isobetanin (Gokhale & Lele, 2014). The degradation mechanisms (deglycosylation, hydrolysis, decarboxylation and dehydrogenation) assume different importance, depending on heat treatment intensity, pH and water activity (Herbach et al., 2006). The hydrolysis leads to the formation of betalamic acid (bright yellow acid) and cycloDOPA 5-O-glucoside (colourless), while the dehydrogenation leads to the formation of neobetanin (yellow). However, in our case the formation of yellow or colourless compounds (absorbances at 470 and 477 nm) was extremely limited; therefore deglycosylation, which in the presence of β-glucosidase leads to the formation of the corresponding aglycones betanin and isobetanin (purple), or decarboxylation, which induces the formation of different decarboxybetacyanins, are more likely. The chromatogram supports the first hypothesis, because aglycones are eluted after their respective glycosides while glycosidic forms have shorter retention times. Paganga and Rice-Evans (1997) reported that polyphenol-glycosides had shorter retention times than have their corresponding aglycones, due to their higher polarities; by comparison with the standards, it is possible to distinguish them by combining retention times and spectral profiles of the individual peaks from the chromatograms. Thus, betanin derivative 1 (peak 12.5 min) could probably be betanidin and betanin derivative 2 (peak 13.3 min) isobetanidin. Overall betanin accounts for approximately 40% of all betacyanins in WB, followed by isobetanin (23%) and, in decreasing order, by the two betanin derivatives.
was not absolute when comparing PME (10.8%) WB with PE (14.9% WB). The betanin derivative 1, scarce in pomace extracts-enriched WB and more abundant in microencapsulate-enriched WB, did not vary much with pomace concentration. The betanin derivative 2 was scarce in PME (10.8% WB) and gradually increased in enriched WB as a function of pomace extracts concentration. Probably the different behaviour of PME (10.8%) and PE-enriched WB for the betanin derivatives is linked to the high protein content of the microencapsulates, which may influence the formation of these compounds. Considering total betacyanin contents, the biscuits with PME 10.8% and PE 14.9% had the highest pigment concentrations, indicating again that microencapsulation contributes to improve betacyanin stability, better preserving their nutritional characteristics.

Fig. 2 Betacyanin contents in water biscuits from bread wheat, einkorn, einkorn-amaranth (70:30), einkorn-buckwheat (70:30) and einkorn-quinoa (70:30), without (0%) or with the addition of extracts (PE 5.7%, PE 10.4% and PE 14.9% of DM pomace) and microincapsulate (PME 10.8% DM pomace) from exhausted red
Finally, the betanin/isobetanin ratio changed markedly after WB baking (from 3.3–5.0 to 1.4–2.1), a phenomenon already observed by Elbe, Schwartz, and Hildenbrand (1981) during red beet juice manufacturing.

### 3.2.3 Total polyphenol content and antioxidant capacity

Fig. 3A shows that microencapsulate-enriched WB had the highest TPC (1883 mg GAE/kg DM), even though they did not have the highest pomace extract concentrations. Therefore microencapsulation better preserved phenolic compounds during WB production. In fact, during cooking, the carrier proteins denature and form a strongly aggregated and protective matrix (Ezhilarasi, Indrani, Jena, & Anandharamakrishnan, 2013). On the other hand, in general, there are no significant differences in TPC among the three PE-enriched WB, and their values (mean: 1480 mg GAE/kg DM) were very similar to the control WB (1443 mg GAE/kg DM).

The PE 5.7%-enriched WB had the lowest antioxidant capacity, together with the control WB (FRAP) or alone (ABTS)
+), while PME showed the highest. Interestingly, WB from buckwheat-added flour were richest in TPC and showed the highest antioxidant capacity, whilst control WB from bread wheat and einkorn flours had the lowest values.

Only in control WBs did the addition of different pomace extract concentrations have a relevant effect on TPC (Fig. 3A) and antioxidant capacity (Fig. 3B and C) while, in pseudocereals-added WB, the influence was evident merely when conveyed through microencapsulates. In a TPC-rich matrix, e.g. buckwheat-added, pomace extract addition may even have the opposite effect.

It has to be remembered that, in addition to total polyphenols, the antioxidant capacity is improved by other compounds, which in this case are mainly betacyanins (Vučić et al., 2014). Consequently, the antioxidant capacity difference between microencapsulate-enriched WB and the other WB is justified not only by their higher TPC but also by their superior betacyanin content and in particular betanin derivative 1. In bread Ezhilarasi et al. (2013) demonstrated that microencapsulated extracts from Garcinia cowa better preserved free hydroxycitric acid than did non-encapsulated extracts.
3.2.4 Heat damage

The addition of pomace extracts in WB led to a reduction of furosine (on average, from 160 to 82 mg/100 g protein; Fig. 3D) and to an increase of HMF (on average, from 1.7 to 4.2 mg/kg DM; Fig. 3E). This behaviour was less evident for the microencapsulate-enriched WB because PME already had a furosine content superior to that of the extracts (Table 2). The HMF content was greater in WB with pseudocereals (on average, 4.0 mg/kg DM) than in the control WB (on average, 1.5 mg/kg DM), probably because the pseudocereals had a higher sugar concentration than had the wheats (Table 1). HMF formation seemed mainly fostered by the sugars in the pomace extracts, and increased as a function of pomace concentration. In fact, in our water biscuits HMF probably came more from sugar caramelization than from the Maillard reaction; this hypothesis is supported by the absence of glycosylisomaltol, whose formation parallels that of HMF (Hidalgo & Brandolini, 2011). Despite the existing differences among samples, HMF concentration was low and comparable to the inferior limits reported by Kocadağlı and Gökmen (2016). Hence furosine appears the most appropriate index for heat damage evaluation under these baking conditions. Overall, the control WB from einkorn and wheat flours showed the lowest heat damage (minimal furosine) while the quinoa-added WB presented the highest.

3.2.5 Colour

The control WB showed the highest \( L^* \) (74.4), the lowest \( a^* \) (2.1) and, together with the WB enriched with PE 14.9%, the lowest \( b^* \) (27.0 and 26.8, respectively) (Fig. 4). The addition of pomace extracts sharply increased the red component (\( a^* \)) and decreased brightness (\( L^* \)). An inverse trend between \( L^* \), which decreased, and \( a^* \), which augmented with increasing pomace percentages, was evident. Bread wheat and einkorn WB were the brightest, while buckwheat-added WB had the lowest \( L^* \), \( a^* \) and \( b^* \). In red beetroot, the red colour originates from betacyanins (Khan, 2015). The deep redness observed in all WB prepared with PME 10.8% indicates a good protection effect of microencapsulation on betacyanins, leading to greater stability compared to other pigments: even after successive extractions with solvents the pomace never discoloured completely.
Conclusions

Pomace additions to water biscuits had a positive effect on betacyanin contents and modified the colour of the end-product. The effects on total polyphenol contents and antioxidant capacity (FRAP and ABTS) in diverse blends varied. In bread wheat and
Einkorn matrices a significant increase, proportional to pomace extract concentrations, was observed. In blends with pseudocereals, richer in antioxidant compounds, the contribution was evident only if conveyed by microencapsulation. The increase of pomace extract in biscuits led to a progressive reduction of furosine and to a slight increase of HMF. The microcapsulate-enriched water biscuits had the highest contents of betanin, isobetanin, total phenolics and antioxidant capacity, thus demonstrating the protective effect of microencapsulation during the production process. Pomace extract, a by-product of juice manufacturing, significantly improved some nutritional characteristics of baked products, especially when conveyed as microencapsulates.

**Uncited reference**

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The authors declare no conflicts of interest.

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Highlights
• Beetroot pomace extracts (PE) and microencapsulated PE (PME) were produced.
• PE and PME were added to pseudocereal-enriched einkorn water biscuits (WB).
• PME-enriched WB were richest in betanin, total polyphenols, antioxidant capacity.
• PE addition generally favoured furosine reduction and HMF increase.
• PE and especially PME improved water biscuits quality.

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