



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

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## ARTICLE INFO

### Chemical compounds studied in this article:

Betanin (PubChem CID: 11953901)

Furosine (PubChem CID: 123889)

Hydroxymethylfurfural (PubChem CID: 237332)

Isobetanin (PubChem CID: 6325438).

### Keywords:

ABTS

Amaranth

Betacyanins

Buckwheat

FRAP

Quinoa

## 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 richest 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.

## 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 tocopherols (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 (Naczek & 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, Lopenen, Klika, & Pihlaja, 2000). Besides polyphenols, beetroot contains betalains, plant pigments which

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couple their strong colouring properties with high antioxidant capacity (Ravichandran, Ahmed, Knorr, & Smetanska, 2012). Betanin (betanidin 5-O- $\beta$ -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 (Stintzing & Carle, 2004). Interestingly, the biologically beneficial properties of betanins 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 Europe 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 (Vulić 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, Stintzing, 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, Alli, & 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. ssp. *monococcum* cv. Monlis), bread wheat (*Triticum aestivum* L. ssp. *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 thresher (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 (Alpha 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 (Laborota Efficient 4000, Heidolph, 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 microencapsulate was obtained by freeze-drying (Alpha 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 (Macrobiotic Prom, Belgrade, 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 cookies formulation, 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 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 – 30% quinoa (Q 30%), 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  $\times$  5.7, Kjeldhal 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 (Rufián-Henares, Delgado-Andrade, & Morales, 2006).

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