Journal of Cereal Science 57 (2013) 312–318

Contents lists available at SciVerse ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

Effect of baking on free and bound phenolic acids in wholegrain bakery products

El-Sayed M. Abdel-Aal*, Iwona Rabalski

Agriculture and Agri-Food Canada, Guelph Food Research Centre, 93 Stone Road West, Guelph, ON, Canada N1G 5C9

ARTICLE INFO

Article history:
Received 7 September 2012
Received in revised form
29 November 2012
Accepted 1 December 2012

Keywords:
Wholegrain bakery products
High-lutein foods
Phenolic acids
Ferulic acid

ABSTRACT

Phenolic compounds, particularly ferulic acid the most abundant phenolic in wheat, are the major contributors to the *in vitro* antioxidant capacity. They are present in wheat in free and bound forms which affect their bioavailability. Thus the current study aims to investigate changes in free and bound phenolic acids occurred during baking in wholegrain bread, cookie and muffin. The products were also fortified with lutein due to its proved health benefits, and were previously evaluated with regard to lutein stability and bioavailability and antioxidant properties. The control and fortified wholegrain bakery products contained reasonable amounts of free and bound phenolic acids with bread products exhibiting the highest level per serving (0.6 and 11.7 mg, respectively). Ferulic acid was the principal phenolic both in the free or bound extracts of the three products followed by *p*-coumaric acid in the bound extracts. Baking resulted in an increase in free phenolic acids in the three products, while bound phenolic acids decreased in bread and slightly changed in cookie and muffin products. Though the effect of baking appeared to be dependent on type of baked product, type of phenolic, recipe and baking conditions, the wholegrain products should be considered good sources of phenolic antioxidants.

1. Introduction

Wholegrain foods are recognized sources of dietary fiber and phenolic antioxidants and their consumption has been linked to the prevention of chronic diseases. Phenolic compounds especially phenolic acids are the main antioxidant contributors in wholegrain products (Abdel-Aal et al., 2012; Kim et al., 2007; Verma et al., 2009) but their composition and content could be altered during processing. It has been reported that processing of cereals or pulses may positively or negatively affect the content of phenolic compounds which possibly impacts their bioactive properties and health benefits (Duodu, 2011). It has been suggested that grain processing may influence ferulic acid uptake in healthy adults (Hamill et al., 2009). In addition, bio-accessibility of ferulic acid from wheat fractions and breads (i.e. the release of phenolic during gastrointestinal or GI transit) using a dynamic *in vitro* system that simulates the upper GI transit and digestion is dependent on the percentage of free ferulic acid (Anson et al., 2009). Only a few studies have investigated effects of baking on phenolic compounds in bakery products.

Wholegrain bread was found to be a much better source of phenolic compounds than white bread and its content of total phenols slightly increased after baking regardless of baking time (10, 20 or 35 min) (Gelinas and McKinnon, 2006). The study also found that the crust of white bread contained a little bit more phenolic compounds than the crumb, perhaps due to Maillard reactions, but this observation was not confirmed in the wholegrain bread. On the contrary, trivial changes in total phenols content and phenolic acids profile caused by baking of control bread or bran-enriched bread have been reported (Menga et al., 2010). Muffin fortified with apple skin as a source of dietary fiber and phenolic compounds was found to retain about 61, 57, 53, 44 and 20% of quercetin glycosides, catechins, chlorogenic acid, phloridzin and cyanidin galactoside, respectively (Rupasinghe et al., 2008). The anthocyanins in apple skin were relatively the most affected compound in comparison with flavonols, dihydrochalcones, phenolic acids and flavan-3-ols.

Recently high-lutein wholegrain bakery products were developed to boost the daily intake of lutein and consumption of wholegrain foods (Abdel-Aal et al., 2010). Baking caused a significant reduction in lutein ranging from 28 to 62% subject to baking conditions and ingredients. Despite the significant losses of lutein in the developed fortified baked products, they still contain a reasonable amount of lutein (up to 1 mg/serving) in addition to the naturally occurring bioactive compounds such as phenolic compounds. Carotenoid losses of 21 and 47% for bread crumb and crust, respectively, have also been reported (Hidalgo et al., 2010).

* Corresponding author. Tel.: +1 226 217 8079; fax: +1 226 217 8181.
E-mail addresses: abdelaale@agr.gc.ca, elsayed.abdelaal@agr.gc.ca (E.-S.M. Abdel-Aal).

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http://dx.doi.org/10.1016/j.jcs.2012.12.001

Bread dough leavening had almost negligible effects on carotenoid losses, while baking resulted in a marked decrease in carotenoids. The bioavailability of lutein in the wholegrain bread, cookie and muffin was also investigated using a fasted and fed digestion model in which food products were subjected to an *in vitro* simulation of human salivary, gastric and duodenal digestion, followed by Caco-2 monolayer absorption (Read, 2011). The fed model resulted in much higher estimates of bioavailability of lutein and the highest fat products (cookie and muffin) resulted in higher overall bioavailability. The high-lutein food products were also found to possess antioxidant potential against oxygen, ABTS and DPPH radicals (Abdel-Aal and Rabalski, in press). The current study is intended to investigate changes in free and bound phenolic acids in the control (unfortified) and lutein-fortified wholegrain bread, cookie and muffin during the baking process. The phenolic compounds in particular ferulic acid are potent antioxidants and their presence in the free or bound form in the wholegrain products would affect their antioxidant properties and bioavailability and eventually their beneficial health effects.

2. Materials and methods

2.1. Baking ingredients

Einkorn wheat (*Triticum monococcum* L.) cultivar AC Knowles was obtained from the Eastern Cereals and Oilseeds Research Centre, Ottawa, ON, Canada. This cultivar is known for its high-lutein content among wheat species (Abdel-Aal et al., 2007). The einkorn wholegrain flour was prepared as described in our earlier study (Abdel-Aal et al., 2010). The wholegrain flour was thoroughly mixed to ensure uniformity and kept at 4 °C until processing and analyses. Other baking ingredients including corn flour, Becel margarine, baking powder, sugar, whey protein isolate and salt were purchased from the retail market in Guelph, ON, Canada. The baking formulas were also fortified with free lutein (Lyc-o-20% oil suspension) kindly provided by LycoRed (Orange, NJ) to produce lutein-fortified bakery products. The level of lutein in the supplement was quantified and confirmed by HPLC (Abdel-Aal et al., 2007).

2.2. Preparation of bakery products

Three wholegrain bakery products (unleavened one-layer flat bread, cookie and muffin) were prepared without and with lutein fortification. Lutein fortification was performed to achieve a level of about 1 mg of free lutein per serving (30 g) in the product. Due to the substantial reduction of lutein during the baking process (Abdel-Aal et al., 2010), the wholegrain flours were fortified with higher levels than the target (about 40–60% more) to compensate for the anticipated loss of lutein during thermal processing. The baking formulas for bread, cookie and muffin, and baking methods using the AACC approved methods or in house procedures were previously described (Abdel-Aal et al., 2010). In the fortified products, lutein was added in the mixing step to ensure uniformity of the formulations. All the freshly baked products were cooled to ambient temperature, then placed in a freezer (−20 °C) and freeze dried. The dried samples were passed through a sieve with 355 μm opening and kept in a freezer at −20 °C until extraction and analyses.

2.3. Analytical tests

Carotenoids in the bakery products were extracted with water saturated 1-butanol and carotenoid extracts were separated and quantified by high performance liquid chromatography (HPLC) using a 1100 Series chromatography system (Agilent, Mississauga, ON) as previously described (Abdel-Aal et al., 2007). The separation was performed on a short C30 column YMC Carotenoid (10 cm × 4.6 mm, packing 3 μm) (Waters, Mississauga, ON). The column was operated at 35 °C and eluted with a gradient mobile system consisting of: (A) methanol/methyl *tert*-butyl ether/nano-pure water (81:15:4, v/v/v) and (B) methyl *tert*-butyl ether/methanol (90:10, v/v) at 1 mL/min. The gradient was programmed as follows: 0–9 min, 100–75% A; 9–10 min, 75–0% A; 10–12 min, hold at 0% A; 12–13 min, 0–100% A; and 13–15 min, hold at 100% A for the short column. The separated carotenoids were detected and measured at 450 nm and the identity of carotenoids was based on the congruence of retention times and UV/Vis spectra with those of pure authentic standards. Four *all-trans* carotenoid standards, lutein and β-carotene (Sigma–Aldrich Canada, Ltd., Oakville, ON), zeaxanthin and β-cryptoxanthin (ChromaDex, Santa, CA) were used for identification and quantification.

Free and bound phenolic acids in wholegrain flours, doughs and products were analyzed by HPLC using the above-mentioned HPLC system equipped with Supelcosil LC C18 column 58298. Free extracts were prepared from a 0.2–0.5 g sample in 5 ml 80% methanol using IKA shaker VXB (IKA Works) for 30 min. The tube content was centrifuged at 10,000 *g* for 10 min, and the extraction was repeated on the residual pellet. Both extracts were pooled together, purged with nitrogen and kept in the refrigerator until further processing and analysis. The residual pellet obtained after removing free phenolic compounds was processed immediately for quantifying the bound phenolic acids. First the left over pellet was washed with hexane, and then centrifuged at 10,000 *g* for 15 min. The hexane supernatant was discarded. A 5 ml of 2 M sodium hydroxide was added to the pellet and the content was purged with nitrogen and mixed on an IKA shaker for 1 h at 70 °C. Then the mixture was cooled and acidified to pH 2 with 2 M hydrochloric acid and centrifuged at 10,000 *g* for 15 min. The acidic supernatant was transferred into a clean separatory funnel. The residual pellet was washed with 10 ml of nano-pure water, then centrifuged at 10,000 *g* and the water supernatant was combined with the acidic supernatant in the separatory funnel. The combined mixture was extracted three times 10 min each time on IKA shaker with 10 ml of ethyl acetate and ethyl ether 1:1 ratio (v/v), then centrifuged and pooled together and eventually dried under nitrogen stream until dryness. The extracted residue was re-dissolved in 5 ml of nano-pure water, filtered through 0.45 μm Acrodisc syringe filter, and stored in a freezer prior to HPLC analysis (Abdel-Aal et al., 2012). Following the completion of bound phenolic acid extracts, free phenolic acid extracts were concentrated and filtered prior to HPLC analysis.

The phenolic acids were eluted at 26 °C using a gradient elution starting with 100% of 6% formic acid and 0% of 6% acidified acetonitrile. The gradient was gradually changed over 35 min to 82% of formic acid and 18% acidified acetonitrile and then kept for 5 min. Additionally, 2 min were allowed to return to the starting conditions. The total run time was 42 min. Separation of phenolic acids was monitored at 5 different channels: 260, 275, 300, 320 and 330 nm. A mixture of 12 phenolic acid standards including gallic, protocatechuic, *p*-hydroxybenzoic, gentisic, 3-hydroxybenzoic, vanillic, caffeic, syringic, *p*-coumaric, ferulic, sinapinic and *o*-coumaric acids was used for calibration, identification and quantification. Typical HPLC chromatograms depicting the separation of free and bound phenolic acids from the standard mix and bread products are presented in Fig. 1, (A) standard mix, (B) unfortified bread bound extract, (C) fortified bread bound extract, (D) unfortified bread free extract and (E) fortified bread free extract. The identity of phenolic acids was confirmed by the iso-absorbance plot analysis and UV absorption bands of phenolic acids (Fig. 1F, G). The