Food Chemistry 203 (2016) 8–15

Contents lists available at ScienceDirect

Food Chemistry

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

Identification of bitter compounds in whole wheat bread crumb

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ARTICLE INFO

Article history:
Received 6 October 2015
Received in revised form 24 January 2016
Accepted 27 January 2016
Available online 28 January 2016

Keywords:
Whole wheat foods
Bitterness
Sensory-guided fraction
Trihydroxyoctadecenoic acids
Crumb

ABSTRACT

Consumer acceptability of whole wheat foods is challenged by negative bitter flavour attributes. In this study, bitter compounds in whole wheat bread crumb were investigated. Utilising sensory-guided multi-dimensional fractionation techniques, the compounds with the highest bitterness intensity in the crumb were purified and identified by LC–MS–ToF and NMR techniques. The main bitter compounds were reported to be *L*-tryptophan, Wessely–Moser isomers apigenin-6-*C*-galactoside-8-*C*-arabinoside & apigenin-6-*C*-arabinoside-8-*C*-galactoside, and 9,12,13-trihydroxy-*trans*-10-octadecenoic acid (pinellic acid). Sensory recombination experiments of the bitter compounds formulated at the concentrations determined in expectorated saliva after bread mastication indicated pinellic acid had the greatest contribution to the bitterness perception of the crumb. Quantitative analysis of pinellic acid in the raw flour was reported to be inherently low compared to bread; the concentration increased more than 30-fold after flour hydration and baking.

1. Introduction

Whole grain foods have been widely recognised as important components of a healthy diet (Liu et al., 1999, 2000; McKeown, Meigs, Liu, Wilson, & Jacques, 2002). Whole grains are rich in essential nutrients such as B vitamins, minerals, polyunsaturated fatty acids, and dietary fibre (U.S. Department of Agriculture & U. S. Department of Health & Human Services, 2010) as well as numerous biologically active components, such as phytochemicals and antioxidants that are considered beneficial to health (Liu & Adom, 2010). Despite the positive benefits associated with whole grain consumption, on average Americans eat less than 1/3 of the USDA recommended intake (U.S. Department of Agriculture & U. S. Department of Health & Human Services, 2010). The low intake of whole grains by children is also recognised as part of the poor dietary habits contributing to the prevalence of child obesity over the past two decades (Ebbeling, Pawlak, & Ludwig, 2002; Institute of Medicine., 2009).

A hurdle to consumption of whole grain food is related to production challenges limiting the development of high flavour quality products. When cereal foods are manufactured with whole grain instead of refined grain flour, lower product acceptability is observed (Bakke & Vickers, 2007; Burgess-Champoux, Marquart, Vickers, & Reicks, 2006; McMackin, Dean, Woodside, & McKinley, 2013). Whole-grain products are commonly associated with bitterness, dryness, astringency, and other negative sensory attributes (Bakke & Vickers, 2007). The compounds responsible for these negative flavour attributes in whole grain foods have been poorly defined, limiting production strategies for product flavour improvement.

Among the limited number of studies focused on characterising bitterness in whole grain foods (Biermann & Grosch, 1979; Heiniö et al., 2008; Heiniö, Liukkonen, Katina, Myllymäki, & Poutanen, 2003), a recent study has (Jiang & Peterson, 2013) reported the Maillard reaction was a main mechanism of bitterness development in wheat bread crust. They identified eight bitter compounds in the bread crust; six were Maillard reaction products that included the tryptophan Amadori rearrangement product (ARP), 5-(hydroxymethyl)furfural (HMF), 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one (DDMP), 4-(2-formyl-5-(hydroxymethyl)-1*H*-pyrrol-1-yl)butanoic acid (PBA), Acortatarin A, and Acortatarin C. Two of the compounds were identified as the amino acid *L*-tryptophan (Trp) and its metabolite tryptophol (TRO). In a follow-up study, the chemical profile of these bitter compounds was investigated in relation to the perceived bitterness of the crust of four commercial whole wheat breads and one refined wheat bread (Bin, Jiang, Cho, & Peterson, 2012). Highly significant correlations ($\alpha < 0.05$) were observed between the perceived bitterness in the crust and the quantity of three Maillard reaction products, specifically 5-(hydroxymethyl)furfural (HMF), 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one (DDMP) and Acortatarin C. These findings provided a chemical fingerprint to profile bitterness in whole wheat products.

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http://dx.doi.org/10.1016/j.foodchem.2016.01.116
0308-8146/

The crumb portion of bread consists of more than 80% of the bread mass and is distinct from the crust, with a higher moisture content and lower cooking temperature during baking. Therefore, Maillard reaction products in the crumb would be distinct from the bread crust, in terms of the chemical species and generation pathways. Consequently, further investigation of the compounds and origin of bitterness in whole wheat foods, specifically in the crumb portion, is warranted. Thus, the objective of the current study was to identify the main bitter compounds in the crumb of whole wheat bread.

2. Materials and methods

2.1. Chemicals

Ethanol (absolute, HPLC grade), methanol (HPLC grade), and formic acid (98–100%, puriss.) were purchased from Fisher Scientific (Fair Lawn, NJ). Linoleic acid–$^{13}C_{18}$ (99 atom% ^{13}C, 97%) was purchased from Sigma–Aldrich (St. Louis, MO). Water was purified through Barnstead Nanopure Diamond water purification system (Thermo Scientific, Dubuque, IA). All other chemicals were of analytical reagent grade from Fisher Scientific (Pittsburgh, PA) and Sigma Chemical Co. (St. Louis, MO).

2.2. Food samples

Bread was made using a modified sponge-dough method (AACC International., 2010). Hard Red Spring (HRS) and Hard White Winter (HWW) flour was obtained from ConAgra Foods (Omaha, NE). In brief, this method involves a two-step process. In the first step, sponge was made by mixing whole wheat flour (478 g), water (350 g), instant dry yeast (7.4 g), yeast food (7.4 g), vital wheat gluten (22 g), and ascorbic acid (20 ppm). The sponge dough was fermented for 3 h at 86°F (30 °C), and then the rest of the ingredients incorporated, including whole wheat flour (257 g), water (185 g), instant dry yeast (2.5 g), salt (17.6 g), sugar (74 g), sodium stearoyl lactylate (1.5 g), and soy oil (25.2 g), to make the dough. The dough was then shaped using a Super-Grain Straight Grain Moulder, proofed at 110/100°F (43/38 °C) to ¼″ (6.25 mm) template, and finally baked at 400°F (204 °C) for 23 min.

2.3. Preparation of bread crumb extract

Five hundred g of HRS bread-crumb was ground in a blender with liquid nitrogen and then extracted by 75% aqueous ethanol (1 L × 3) at room temperature for 16 h. The extract was centrifuged at 8000 rpm for 10 min at 5 °C; supernatant was freed from ethanol under vacuum, and freeze-dried using a Virtis Freezemobile 35 EL (SP Scientific, Warminster, PA). For further sample clean up, the powder from the freeze-dried sample was re-solubilised with 120 mL of ethanol: water (20:80 *v*/*v*) and two 60-mL aliquots were passed through a 12-g preconditioned C18 SPE cartridge (Supelco, Bellefonte, PA). The eluent was pooled (hydrophilic portion) and the SPE further eluted with 30 mL of 100% ethanol (hydrophobic portion). Both hydrophilic and hydrophobic eluents were individually concentrated under vacuum to yield 40 mL of crumb extract and then filtered through 0.45-μm nylon syringe filter (Millex; Millipore, Billerica, CA). Further fractionation was performed by RP-HPLC techniques.

2.4. Preparative HPLC fractionation – first dimension

The study of bitterness in bread crust (Jiang & Peterson, 2013) reported that in the bread crust, bitter substances were largely presented as hydrophobic compounds with small molecular weight (MW < 1000). Therefore, for initial fractionation of the whole wheat bread crumb extract an RP-HPLC column was selected for analysis. A preparative HPLC system consisting of a binary pump system (Shimadzu LC-8A), a manual injector (Rheodyne 3725i), a UV/Vis detector (Shimadzu SPD-10Ai), and a fraction collector (Shimadzu FRC-10A) was used for fractionation. Five ml aliquots were separated using a preparative Agilent Pursuit® C18 column (21.2 × 150 mm, 5 μm). The mobile phase was maintained at a flow rate of 15 mL/min using a binary solvent system of 0.1% formic acid in water (**A**) and methanol (**B**). The elution gradient started at 10% **B** (0–5 min), linearly increased to 30% **B** (5–10 min), then to 50% **B** (10–20 min), then ramped 80–100% **B** (20–24 min), held at 100% **B** (24–26 min), and re-equilibrated at 10% **B** (26–40 min). The absorbance of the UV/Vis detector was set at 280 nm. The effluent was collected in 26 fractions. Fractions were collected from eight runs, individually combined and freeze-dried twice to completely remove the solvent. The resulting residues obtained were then dissolved in 15 mL water, and each fraction was evaluated by a trained sensory panel for bitterness intensity. The dose for evaluation was 1 mL, which was equivalent to extracts from 25 g of bread sample. The fractions with the highest bitter intensity were identified as #11, #16, and #21–25 and are shown in Fig. 1. Based on LC/MS analysis, Fraction #11 (Fig. 1) was easily identified as *L*-tryptophan, a known bitter amino acid, and further confirmed by ^{1}H NMR.

2.5. Semi-preparative purification of bitter fractions – second dimension

Targeted bitter fractions #16 and #21–25 (Fig. 1) from the first dimensional fractionation were subsequently further purified by a second HPLC separation, evaluated by the bitterness screening panel and the primary bitter regions analysed by LC/MS, which identified the tentative structures in those fractions. Fraction #16 (Fig. 1) contained a mixture of flavonoid-*C*-glycoside isomers, which was further fractionated by preparative HPLC on a Agilent Zorbax® Bonus-RP column (21.2 × 100 mm, 5 μm) using a binary solvent system of 0.1% formic acid in water (**A**) and acetonitrile (**B**) at a flow rate of 10 mL/min. Separation of flavonoid-*C*-glycoside isomers that co-eluted from the first dimension was successfully achieved by applying gradient elution from 10% **B** to 30% **B** (7–27 min). Purity of the flavonoid-*C*-glycoside isomers was >96% as determined by NMR. Fractions #21–25 (Fig. 1) were determined by LC/MS to be hydroxylated fatty acids. In fractions #22 and #23 a major component (ESI^- *m*/*z* 329) was present in both fractions with co-elutions of its isomer (ESI^- *m*/*z* 329) and oxidised product (ESI^- *m*/*z* 327). Separation of hydroxylated fatty acids in fractions #22–23 was performed on a semi-preparative Restek Pinnacle C18 column (10.0 × 250 mm, 5 μm; Restek, Bellefonte, PA) using a binary solvent system of 0.1% formic acid in water (**A**) and acetonitrile (**B**) at a flow rate of 3.5 mL/min. Gradient elution was applied from 50% to 80% **B** (4–20 min). Since these hydroxylated fatty acids are non-active UV/Vis compounds, on-line mass-directed fractionation was achieved by using a semi-preparative HPLC–MS system consisting of a Shimadzu® HPLC pumping system and a Waters® ZQ mass spectrometer. A minor portion of effluent was split into the MS detector and the remainder was sent to the fraction collector. Collection of major hydroxylated fatty acids by MS was obtained by triggering the collection of the *m*/*z* 329 and 327 under ESI^-. The purified fractions were freeze-dried twice to completely remove solvent. The isolates were dissolved in 5% aqueous ethanol solution, passed through a 5-g preconditioned C18 cartridge (Supelco, Bellefonte, PA), eluted with ethanol, and then diluted with water to <5% ethanol and freeze-dried twice.