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Changes in the phytochemical profile of rye bran induced by enzymatic bioprocessing and sourdough fermentation

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ABSTRACT

Rye bran is a rich source of phytochemicals, such as alkylresorcinols, benzoxazinoids, and phenolic acids, which likely are attributing to the positive health effects of whole-grain foods. In this study, we examined the effect of two types of bioprocessing of rye bran on the phytochemical profile as compared with non-processed rye bran, using a non-targeted LC–MS metabolomics method. The four breads included in the study were commercial sourdough rye bread baked with 100% rye flour, a white wheat bread, a white wheat bread fortified with native rye bran, and a white wheat bread fortified with bioprocessed rye bran. The changes induced by the combination of enzymatic processing and yeast fermentation in the phytochemical pool were dissimilar to sourdough fermentation. Notably, the amount of free phenolic acids in bran was significantly increased, some of the hexose moieties were released from benzoxazinoids, and alkylresorcinols experienced moderate degradation. The enzymatic bioprocessing increased the bioaccessibility of several phytochemicals, thus making breads fortified with bioprocessed rye bran attractive targets of functional food development.

1. Introduction

Phytochemicals are plant-derived compounds that may have biological activity as part of the human diet but are not used by the body as nutrients. Whole grain, which contains the principal anatomical components of the cereal grain in their original proportions, is a major source of phytochemicals in the human diet. This is not only due to the high concentration of several bioactive compounds in the bran and germ (Liu, 2007), but also because cereals are the most important dietary source worldwide in terms of energy intake (Bruinsma, 2003).

Rye (*Secale cereale* L.) is one of the four major cereals produced and consumed in Northern Europe, along with wheat, barley, and oats. Rye breads are predominantly prepared from wholemeal flour using sourdough fermentation, which enables the flour to be properly processed and enhances the flavour, texture, and shelf life of the bread (Katina et al., 2005). It contains a significant amount of phytochemicals from various chemical groups, including phenolic acids, lignans, and alkylresorcinols, mainly in the outer parts of the cereal grain (Bondia-Pons et al., 2009). While phenolic acids are present even in the starchy endosperm of the rye kernel, alkylresorcinols are located exclusively in the bran section of wheat and rye, thus making them potential biomarkers of whole grain intake (Landberg, Kamal-Eldin, Salmenkallio-Marttila, Rouau, & Åman, 2008; Ross, Åman, & Kamal-Eldin, 2004). Additionally, benzoxazinoids have recently been identified from whole-grain rye (Hanhineva et al., 2011; Pedersen, Laursen, Mortensen, & Fomsgaard, 2011) and discovered to be bioavailable dietary metabolites in humans (Adhikari et al., 2013; Beckmann et al., 2013; Hanhineva et al., 2014).

The most abundant phenolic compounds in rye are phenolic acids, particularly ferulic acid (Andreasen, Christensen, Meyer, & Hansen, 2000). In unprocessed (native) rye bran, phenolic acids are a part of the dietary fibre complex of the bran, cross-linking polysaccharides and thus strengthening the insoluble cell wall structures (Faulds & Williamson, 1999). In particular, ferulic acid seems to be mostly ester-bound to heteroxylans, major dietary fibre components of bran (Andreasen et al., 2000), thus being for the most part unavailable for absorption in the GI tract. In fact, free phenolic acids compose <4% of the total phenolics in native rye grain (Nyström et al., 2008). Bran bioprocessing – either by the sourdough process or enzymatic treatment – causes partial degradation of the cell walls; specifically the activity of ferulic acid esterase releases phenolic compounds from the fibre matrix of the bran, thus improving their bioavailability (Anson et al., 2009; Katina et al., 2007). Alkylresorcinols, however, are more resistant to food processing, slightly reducing in concentration after sourdough fermentation and having a slight increase in concentration after fermentation of bran from native grains (Katina et al., 2007). In the sourdough fermentation, lactic acid bacteria have strain-dependent capabilities for conversion of phenolic compounds, such as hydrolytic cleavage of ester-

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linked sugars from flavonoids and ferulic acid, and decarboxylation of hydroxybenzoic and hydroxycinnamic acids (Gänzle, 2014). Fermentation also activates endogenous enzymes in the grain. These metabolic conversions are likewise increasing the bioavailability of phenolic compounds.

The biological activity of dietary phytochemicals, likely arising from the synergistic and antagonistic effects on various metabolic processes, may attribute to the beneficial health effects of diets rich in whole grains and other plant-based foods, as has consistently been observed in epidemiological studies (2013; Liu, 2004; Slavin, Jacobs, Marquart, & Wiemer, 2001). The bioavailability, metabolism, and potential health effects of these groups have been frequently reviewed (Andersson, Dimberg, Åman, & Landberg, 2014; Bartłomiej, Justyna, & Ewa, 2012; Bondia-Pons et al., 2009; Borneo & León, 2012; Okarter & Liu, 2010; Slavin, 2003). High intake of whole-grain rye has been associated with increased satiety (Isaksson, Fredriksson, Andersson, Olsson, & Åman, 2009) and improved acute insulin response possibly due to enhanced pancreatic β cell function (Juntunen, Laaksonen, Poutanen, Niskanen, & Mykkanen, 2003). In mice, whole-grain rye has been observed to reduce body weight, improve insulin sensitivity, and decrease the level of total plasma cholesterol, as compared with whole-grain wheat (Andersson et al., 2010).

Despite its positive health implications, whole-grain rye bread has certain sensory attributes that may decrease its consumer acceptance, such as sourness and the bitter and intense flavour, which are strengthened by the sourdough process (Heiniö, Liukkonen, Katina, Myllymäki, & Poutanen, 2003). Furthermore, the high fructan content in rye – both in the endosperm and bran sections – may trigger abdominal symptoms in people with irritable bowel syndrome (Frølich, Åman, & Tetens, 2013; Rakha, Åman, & Andersson, 2010; Shepherd, Parker, Muir, & Gibson, 2008). Therefore, it may be sensible to develop products that combine the generally more palatable taste and in some cases better digestibility of another type of bread, such as white wheat bread, with the high content of fibre and phytochemicals in rye bran.

Liquid chromatography coupled with mass spectrometry (LC–MS) is the prevailing method for the analysis of phytochemicals, owing to its high sensitivity and accuracy, as we have reviewed recently for whole grain phytochemicals (Koistinen & Hanhineva, 2015). The mass spectrometry–based knowledge of phytochemicals present in the major cereals encompasses all the main groups of grain phytochemicals; reverse-phase LC–MS is well-suited for detecting compounds with semi-polar characteristics which are present in several phytochemical classes. >5000 phytochemicals have been identified from edible plant products (Liu, 2013) with a steadily increasing number of novel compounds reported alongside improvements in the sensitivity of analytical approaches. The knowledge is yet far from complete, however, as numerous detectable compounds lack structure elucidation, available reference compounds, or published data on MS/MS fragmentation, essential for the identification of phytochemicals in biological samples containing thousands of compounds.

In this study, the aim was to compare the phytochemical profiles of two differently processed breads rich in rye bran: a bread with rye bran processed by enzyme-aided yeast fermentation added in wheat matrix and a traditional sourdough fermented wholemeal rye bread. In addition, we aimed to compare these breads with a white wheat bread fortified with native rye bran and plain white bread. Furthermore, one purpose of the study was to increase the knowledge of rye bran phytochemicals by providing mass spectrometric data on the identification of previously known and potentially novel phytochemicals present in rye bran.

2. Materials and methods

2.1. Breads

The test breads used in this study were acquired and processed as described by Lappi et al. (2013) and Nordlund, Katina, Aura, and Poutanen (2013). Briefly, four breads were included: a commercial whole-grain rye bread baked with 100% rye flour (R bread; Vaasan Oy, Finland), a white wheat bread (WW bread), a white wheat bread fortified with native rye bran (RB + WW bread), and a white wheat bread fortified with bioprocessed rye bran (BRB + WW bread). The rye bran, provided by Fazer group (Lahti, Finland), was milled and air-classified to reduce the starch content. The enzymatic bioprocessing of the rye bran was carried out using a hydrolytic enzyme mixture containing Depol 740L (Biocatalysts, UK; dosing 200 nkat/g bran, based on xylanase activity), containing ferulic acid esterase activity, and Grindamyl A 1000 (Danisco A/S, Denmark; dosing 75 nkat/g bran, based on xylanase activity) at 65% water content in 40 °C for 4 h, after which baker's yeast (1.25%; Sunnuntai, Finland) was added and the mixture was fermented in 20 °C for 20 h. For preparing the BRB + WW and RB + WW breads, 35% of the white wheat flour was replaced with the wet and dry bran samples for BRB + WW and RB + WW, respectively, based on dry matter. The amount of rye bran was adjusted to have the breads contain similar amounts of total dietary fibre (10–13%). The breads were freeze-dried prior to the sample preparation.

2.2. Sample preparation

Small slices from each test bread were frozen with liquid nitrogen and ground into fine powder using a TissueLyser II bead mill (Qiagen, Hilden, Germany). Four replicates were made from each sample by measuring approximately 100 mg of the powdered sample and adding 300 μl of 80% methanol in water (v/v) per 100 mg of sample as the extraction solvent. The samples were then shaken in room temperature for 15 min, centrifuged for 5 min at +4 °C and 16,249 × *g*, and finally filtered using syringe filters (Acrodisc CR 13 mm syringe filter with 0.2 μm PTFE membrane; PALL, Port Washington, NY, USA).

2.3. HPLC–MS experiment

The HPLC–MS analysis was carried out as described by Hanhineva et al. (2014). The liquid chromatography was performed on a 1290 Infinity Binary UPLC system (Agilent Technologies, Santa Clara, CA, USA). For the reversed-phase separation, a Zorbax Eclipse XDB-C18 column (Agilent Technologies) with dimensions of 2.1 × 100 mm and a particle size of 1.8 μm was used. The column temperature was 50 °C, the flow rate was 0.5 ml/min, the injection volume was 2 μl and the sample tray temperature was +4 °C. The gradient elution consisted of water (solution A) and methanol (solution B), both containing 0.1% (v/v) formic acid. The mass spectrometric analysis was performed in an Agilent 6540 Q-TOF (quadrupole time-of-flight) with a Jet Stream (electrospray ionisation) ion source in the negative ion mode. Nitrogen was used as the collision cell gas and as the ion source. The MS/MS analyses were carried out automatically by using a fragmentor voltage of 100 V, scan range of 20–1600 *m/z* and a precursor isolation width of 1.3, and selecting 4 most abundant precursor ions for fragmentation. The collision energies for MS/MS analysis were chosen as −10, −20 and −40 eV, for compatibility with the METLIN database. Due to corrupted data, one BRB + WW replicate was excluded from the following steps.

2.4. Data analysis

The initial search for compounds by their molecular features was carried out using MassHunter Qualitative Analysis version 7.0 (Agilent Technologies). The extraction mode was set to small molecules with a retention time window at 0.45–15.5 min, mass peak threshold at 300 counts, and absolute compound peak height at 3000 counts. The allowed negative ion species were singly charged ions and chloride adducts. The peak spacing tolerance for isotope grouping was selected as *m/z* 0.0025 plus 7 ppm, with an isotope model for common organic