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Bread from common cereal cultivars contains an important array of neglected bioactive benzoxazinoids

Hans Albert Pedersen, Bente Laursen, Anne Mortensen, Inge S. Fomsgaard *

Department of Integrated Pest Management, Faculty of Agricultural Sciences, Aarhus University, Forsøgsvej 1, DK-4200 Slagelse, Denmark

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ABSTRACT

Bread is consumed in large quantities all over the world, and rye bread is especially popular throughout the Nordic countries. Wholemeal bread is highly recommended as a basic ingredient in daily food, because wholemeal food products generally promote good health due to their vitamin, mineral and fibre content. The literature suggests that wholemeal products have other health-promoting effects even if the ingredients responsible have not been identified. Benzoxazinoids are a group of natural products that have not previously been reported in mature grains. Here, we report for the first time the identity and quantity of 10 compounds of the benzoxazinoid family in mature grains, hydrothermally processed grains of durum wheat (*Triticum durum*, cv. Kamut), a commercial variety of rye (*Secale cereale* cv. Picasso) and an old Nordic rye landrace (*S. cereale*, Svedjerug), as well as in bread baked with flour milled from those grains. Concentrations of the 10 benzoxanoids were determined using LC–MS/MS and ranged from 0 to 348 nmol g^{-1} for conventional flour, to 772–1177 nmol g^{-1} in bread baked with flour from hydrothermally processed grains and to 3116–5570 nmol g^{-1} in flour from hydrothermally processed grains. Benzoxazinoids possess documented physiological effects, and research into the importance of these compounds in the daily diet is therefore needed. Ongoing studies in our lab on the uptake and transformation of benzoxazinoids in mammals will be reported in the near future.

1. Introduction

Benzoxazinoids are allelopathic compounds exuded from the roots of several crops including rye and wheat. Rye and wheat are staple crops for human consumption as they are used worldwide in bread-making. Benzoxazinoids include benzoxazolinones such as 2-benzoxazolinone (BOA) and 7-methoxy-2-benzoxazolinone (MBOA), lactams such as 2-hydroxy-1,4-benzoxazin-3-one (HBOA) and 2-hydroxy-7-methoxy-1,4-benzoxazin-3-one (HMBOA) and their respective glucosides, as well as hydroxamic acids such 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA) and 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and their corresponding glucosides. Benzoxazinoids are known from maize and wheat (Sicker, Frey, Schulz, & Gierl, 2000), as well as rye. The occurrence of benzoxazinoids in cereals at several growth stages was recently investigated (Krogh et al., 2006; Mogensen, Krongaard, Mathiassen, & Kudsk, 2006; Rice, Park, Adam, Abdul-Baki, & Teasdale, 2005; Stochmal, Kus, Martyniuk, & Oleszek, 2006), as was their importance as alternative means of suppressing weeds and diseases (Etzerodt, Mortensen, & Fomsgaard, 2008; Fomsgaard, 2006; Krogh et al., 2006; Søltoft, Jørgensen, Svensmark, & Fomsgaard, 2008). Several studies have focused on a small complement of benzoxazinoid compounds (Copaja, Nicol, & Wratten, 1999; Copaja, Villarroel, Bravo, Pizarro, & Argandoña, 2006; Katina et al., 2007; Zúñiga, Argandoña, Niemeyer, & Corcuera, 1983), including just one report mentioning non-specific total benzoxazinoid content from mature cereal grains (Katina et al., 2007).

Food preparation including the hydrothermal processing (HTP) of cereal grains (e.g. extrusion cooking, parboiling, germination, sprouting, malting, or fermentation) is becoming more and more common. Throughout this paper HTP will refer to the process of controlled consecutive wetting and drying of grains. Bread is consumed in large quantities all over the world, especially rye bread throughout the Nordic countries. Considering that DIBOA has been known for some time to inhibit cancer cell growth *in vitro* (Habib, Ross, Lewenstein, Zhang, & Jaton, 1995; Roberts et al., 1998; Zhang et al., 1995), our present discovery of an array of benzoxazinoids in cereal grains therefore indicates a need for detailed studies of these compounds. This study aimed to map quantitatively the presence of 10 benzoxazinoid compounds in mature grains of selected cultivars of rye and wheat before and after five-day HTP treatment, as well as in bread baked with the corresponding HTP and conventional flours.

* Corresponding author. Tel.: +45 89 99 36 10.
E-mail address: inge.fomsgaard@agrsci.dk (I.S. Fomsgaard).

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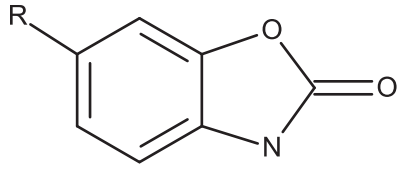
2. Materials and methods

2.1. Reagents

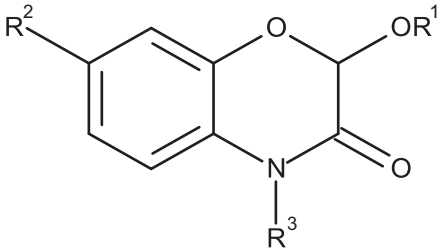
Analytical grade acetonitrile and methanol were obtained from Rathburn (Walkerburn, Scotland) and glacial acetic acid from Baker (Deventer, The Netherlands). Benzoxazinoid standards (structures shown in Fig. 1) were obtained as gifts or were synthesised (Carlsen et al., 2009).

2.2. Grain and flour samples

Mature grains of the commercial durum wheat cultivar Kamut and the rye landrace Svedjerug were purchased from Aurion (Hjørring, Denmark). Rye grains of the commercial cultivar Picasso were purchased from Lochow-Petkus GmbH (Bergen–Wohlde, Germany). The grains (including the bran) were milled on a Fidibus 21 milling machine (KoMo GmbH, Otzberg–Lengfeld, Germany) into wholemeal flour. Xtra brand commercial wheat flour containing only the endosperm was purchased from Coop Denmark (Albertslund, Denmark). As it contained only insignificant amounts of benzoxazinoids, the Xtra brand commercial wheat flour was used as a blank flour sample.



	R	
Benzoxazolinones	H	BOA
	OCH_3	MBOA



	R^1	R^2	R^3	
Lactams	H	H	H	HBOA
	H	OCH_3	H	HMBOA
	Glc	H	H	HBOA-Glc
	Glc	OCH_3	H	HMBOA-Glc
Hydroxamic acids	H	H	OH	DIBOA
	H	OCH_3	OH	DIMBOA
	Glc	H	OH	DIBOA-Glc
	Glc	OCH_3	OH	DIMBOA-Glc

Fig. 1. Structures of the 10 benzoxazinoids quantified.

HTP flour was produced by placing 20 g of seeds in each of 12 trays stacked in sets of four. The trays contained holes to allow water to slowly run through but retain the seeds. The seeds were then wetted with 300 ml water on the morning of day one, and the water allowed to run through before being collected and discarded. In the evening, the procedure was repeated using only 200 ml water. The wetting procedure was repeated once every morning and once every evening using 200 ml water for a total of eight more times over the next 4 days. The total HTP treatment time was thus 5 days; the HTP grains were subsequently collected, dried in an oven at 50 °C and milled into flour. The water content of the resulting flour was determined by drying 1 g of each flour sample overnight at 105 °C; this step was performed in triplicate. In this study, flour milled from HTP grains, as well as bread baked using flour milled from HTP grains, are for brevity denoted HTP flour and HTP bread, respectively.

2.3. Bread samples

Bread prepared for this study was either conventional bread or HTP bread. Conventional bread made from each cereal was baked using 1/3 (175 g) blank wheat flour and 2/3 (350 g) conventional flour. HTP bread made from each cereal was baked using 1/3 (175 g) blank wheat flour, 1/3 (175 g) conventional flour and 1/3 (175 g) HTP-flour. For each bread 300 ml of water, one teaspoon of salt and two teaspoons of dry yeast were used.

In total, seven loaves of bread were prepared; of these, three were loaves of HTP bread (each with one of the three cereals) and three were loaves of conventional bread (one for each cereal). A control bread was also prepared using only blank wheat flour.

Each bread was baked in the same manner, i.e., using a standard commercial baking machine set to the standard wholemeal program for a total preparation time (including kneading, raising and baking) of 3 h 40 min. The temperature profile applied by the machine during baking began at room temperature for 35 min before increasing from 36 to 40 °C over 125 min and finally baking at 160 °C for 1 h. Kneading took place during the first 5 min and between 35 and 55 min. The temperature of the bread lagged approximately 10 min behind the oven. Upon baking, the bread was allowed to cool. Two slices were cut from each loaf and stored at −18 °C until preparation for analysis. The water content was determined by recording the weight of each slice before and after freeze-drying (see Section 2.4.).

2.4. Preparation and extraction of bread samples

Each frozen bread slice was lyophilised and homogenised to a powder in a Waring (Herlev, Denmark) blender. 0.1 g of each sample was removed for extraction on a Dionex ASE 350 Accelerated Solvent Extractor (Hvidovre, Denmark) as follows: to each 33 ml extraction cell was added a cellulose filter, 5 g of inert Ottawa sand, 0.1 g of prepared bread sample, another 5 g of inert Ottawa sand and another cellulose filter. The remainder of each cell was filled with glass beads, and the samples were then extracted using the following settings: temperature, 80 °C; heat, 5 min; static time, 3 min; cycles, 4; rinse volume, 60%; purge, 60 s. The extraction solvent contained 19% water, 80% methanol and 1% acetic acid (v/v).

2.5. LC–MS analysis of bread extracts

Bread extracts were analysed in multiple reaction mode (MRM) on an Applied Biosystems 3200 Q Trap LC–MS (Nærum, Denmark). The instrument parameters were as follows: curtain gas, 12 psi; CAD, medium; temperature, 475 °C; GS1, 60 psi; GS2, 60 psi;