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Flavour and stability of rye grain fractions in relation to their chemical composition

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ABSTRACT

Wholegrain rye is health-beneficial, but intensive and bitter flavour of rye foods, supposedly caused by specific phenolic compounds and small peptides, restricts its use. The aim was to study the stability and sensory quality of rye grain fractions in relation to their chemical composition. In addition to distinctive sensory and chemical properties of wholegrain flour, endosperm-rich fraction and two differently prepared brans, the rye fractions had differences in their stability. The endosperm fraction had the poorest stability and mildest flavour. The rancid flavour was related to free fatty acids. The bran, intense and bitter in flavour, but more stable, had the highest content of phenolic compounds. The high content of phenolic compounds of bran contributing to its bitterness probably also improved its stability by reducing the formation of flavour-active lipid-derived oxidation products. Stability of the rye bran made by an air-classification process was inferior to the bran made by a conventional sieving process, despite similar content of phenolic compounds. Changes in the development rate of rancidity and intensity of flavour were faster in the air-classified than in the sieved bran. This could be due to different microstructure and particle size of the brans, influencing oxidation susceptibility.

1. Introduction

Wholegrain cereal foods are health-beneficial, which is presumed to relate to the high content of bioactive phytochemicals and dietary fibre of grains (Okarter & Liu, 2010; Vitaglione, Napolitano, & Fogliano, 2008). Hence, increase in the consumption of wholegrain foods is generally supported by health authorities. Consumers' food choices are, however, mostly driven by taste, and healthiness is perceived as an additional positive element (Verbeke, 2006). Certain intrinsic flavours in wholegrain foods may restrict their use by some consumers. For example, the intense and bitter taste of rye, suggested to be caused at least by specific phenolic compounds and small peptides (Heiniö et al., 2008; Heiniö, Nordlund, Poutanen, & Buchert, 2012), may form an obstacle for the use of rye in food products.

Cereal flavour is formed by a complex mixture of volatile and non-volatile chemical compounds in different relative concentrations. Volatile compounds, such as aldehydes, ketones and alcohols, are known to influence flavour, causing for example green, sweet and fruity odour notes (Heiniö, Liukkonen, Katina, Myllymäki, & Poutanen, 2003; Kirchhoff & Schieberle, 2002). Non-volatile compounds, i.e. amino acids, small peptides, sugars and phenolic compounds are reported to contribute to cereal flavour by causing bitter notes (Dimberg, Molteberg, Solheim, & Frölich, 1996; Heiniö et al., 2008, 2012). The flavour attributes are unevenly distributed in the grain. In rye grain, the innermost endospermic part is mild-tasting, whereas the outermost bran fraction which also contains significant amounts of bioactive compounds (Liukkonen et al., 2003) is perceived bitter (Heiniö et al., 2003).

Lipid- and protein-derived compounds play a particularly important role in the formation of flavour of cereal products and are typically also related to changes in the cereal flavour during storage. Formation of bitter peptides from proteins may be initiated, for example, during grain processing by activation of endogenous proteolytic enzymes of grain (Brijs, Bleukx, & Delcour, 1999), whereas aldehydes are typical secondary lipid oxidation products known to have an impact on cereal flavour (Grosch & Schieberle, 1997). The reactions between secondary lipid oxidation products and amino acids lead to the formation of carbonyl compounds, which have an effect on the cereal food flavour (Gardner, 1979). Deterioration of cereal flavour during storage is typically related to the oxidation and hydrolysis of lipids, which cause rancid flavour in a product. An increase in the amount of free fatty acids (FFA) increases the risk of rancidity since the FFAs are precursors for various flavour compounds (Forss, 1969). Oxidative rancidity develops as a result of enzymatic or non-enzymatic processes, and the hydrolytic rancidity is a result of lipase activity (Welch, 1995). Endogenous lipase, lipoxygenase and

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peroxidase enzymes related to these reactions of lipids are often activated by the physical disruption of the grain, e.g. by milling.

Since oat grain has relatively high lipid content (lipid content of oat is around 6%, whereas the lipid content of rye is only around 2–3%), and it also contains lipolytic enzymes (Matlashewski, Urquhart, Sahasrabudhe, & Altosaar, 1982), studies on the formation and prevention of rancid flavour have been focused mainly on the oat products (Heiniö, Lehtinen, Oksman-Caldentey, & Poutanen, 2002; Heiniö, Oksman-Caldentey, Latva-Kala, Lehtinen, & Poutanen, 2001; Lehtinen, Kiiliäinen, Lehtomäki, & Laakso, 2003). Combined germination and heat treatment of oat has been shown to decrease the formation of rancidity (Heiniö et al., 2002). The heat treatment has been proved to inactivate the lipases and oxidases present in oat grain (Ekstrand et al., 1993; Molteberg, Magnus, Bjørge, & Nilsson, 1996). Added or endogenous phenolic compounds, such as avenanthramides and phenolic acids, have also been reported to delay lipid oxidation, and thus prevent the development of rancidity (Molteberg, Solheim, Dimberg, & Frölich, 1996).

Fractionation of grain is generally used to remove the outer layers to improve the technological properties of flour, but also to decrease the intensity of the flavour. For example, milling fractionation of rye has been reported to produce different sensory profiles of both flour and bread (Heiniö et al., 2003). Peeling of grain is also used to remove the germ to avoid lipid oxidation and formation of rancid flavour in flour. By removing the outermost layers of grain, also the content of health-beneficial compounds, such as dietary fibre and phytochemicals, is decreased. However, the “shorts” milling fraction of rye, with relatively high content of phytochemicals, has been shown to contain cereal-like but not bitter flavour in both flour and bread (Heiniö et al., 2003).

In the present work the objective was to study the stability and flavour profile of different fractions of rye grain in relation to their chemical composition.

2. Experimental

2.1. Raw materials

Rye kernels (cultivar Amilo, falling number 264) were obtained from Kesko Corporation (Helsinki, Finland). All chemicals, solvents and reagents were of analytical grade unless specified otherwise.

2.2. Mechanical fractionation of rye

For fractionation of rye by air-classification, rye kernels were first milled at Fazer Mill & Mixes (Lahti, Finland) to obtain commercial medium-coarse wholegrain rye flour R1 (mean particle size 59 ± 4 μm). This wholemeal rye flour R1 was fractionated using a consecutive milling and air-classification process to produce a coarse rye fraction R4 (mean particle size 105 ± 3 μm, later in the text stated as *bran R4*) and fine rye fraction R3 (mean particle size 33 ± 3 μm, later in the text stated as *endosperm R3*) (Fig. 1A). To compare conventional rye bran to the air-classified bran fraction, rye kernels were also milled using progressive milling to prepare a coarse fraction, i.e. the conventional rye bran (mean particle size 144 ± 4 μm, later in the text *bran R7*) (Fig. 1B). Parameters and the yields of the fractionation processes are shown in Table 1. The prepared rye fractions R1–R7 (Fig. 1A and B) were then milled with Alpine UPZ 100 flour mill, using 18 000 rpm and a 0.3 mm sieve.

2.3. (Bio)chemical and physical characterization of rye fractions

Moisture content was determined by weighing the samples before and after oven drying (AOAC method 14.004, 1980; AOAC method 7.007, 1980). *Ash content* was analysed by weighing the samples before and after burning at 500 °C overnight (17 h). *Protein content* was calculated by formula 6.25 × nitrogen for which the nitrogen content was determined by Kjeldahl method, using a Kjeltec Auto 1030 analyser (AOAC method 14.068, 1980). Protein hydrolysis was estimated based on the detection of *free amino nitrogen* (*FAN*) by the spectrophotometric method (Analytica EBC, 1998). *Total fat* content was determined gravimetrically by the Foss Soxtec method, using diethyl ether as a solvent (AOAC method 922.06, 2005). For lipid class separation the extraction was done using modified Folch in which the chloroform is replaced with dichloromethane (Cequier-Sánchez, Rodríguez, Ravelo, & Zarate, 2008). *Polar lipids*, *diglycerols*, *triglycerols*, *free fatty acids* and *degree of fatty acid unsaturation* (*DUS*) were analysed according to Liukkonen, Montfoort, and Laakso (1992). Prior to TLC separation samples were supplemented with the internal standards (1,2-dipentadecanoyl-sn-glycero-3-phosphocholine, 1,3-diheptadecanoin, triheptadecanoin and heptadecanoic acid). The fatty acid composition of each lipid class was analysed as their methyl esters. DUS values of the samples were calculated based on the composition of the fatty acids as follows: DUS (Δmol^{-1}) = Σ [(% mono-unsaturated fatty acids) + 2 × (% fatty acids with two double bonds) + 3 × (% fatty acids with three double bonds)] / 100. In addition, in order to evaluate the endogenous lipid-modifying enzyme activities, lipid class composition was also analysed after treating samples in excess water for 15 h before the lipid extraction as described in Lehtinen et al. (2003).

The content of *total carbohydrates* was calculated by the following formula: total carbohydrates (g/100 g FW) = 100 − moisture (%) − protein content (%/FW) − crude fat (%/FW) − ash (%/FW). *Soluble and insoluble dietary fibre* was analysed according to the AOAC method 991.43 (2005). *Soluble and total pentosans* were analysed according to the colorimetric method as described by Douglas (1981), and *β-glucan* according to McCleary and Codd (1991) using the mixed-linkage beta-glucan kit (Megazyme, Ireland). *Reducing sugars* were analysed by the DNS method according to Bernfeld (1955). *Free glucose* was determined based on the total starch assay kit (Megazyme, Ireland, AOAC Method 996.11, with improvements). *Total and free phenolic compounds* were analysed according to Singleton and Rossi (1965), using the Folin–Ciocalteu method, and for the detailed analysis of phenolic compounds (phenolic acids, alk(en)ylresorcinols (ARs), benzoxazinoids, and flavonoids) was performed by HPLC-DAD according to Pihlava et al. (submitted for publication). All chemical analyses were performed in two replicates.

Antioxidant activity of the fractions was estimated *in vitro* by using DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay, and by assessing the capability to inhibit lipid oxidation in liposome model system. For the *DPPH radical scavenging assay* flour samples of 5 mg, 20 mg and 50 mg (as powdered dry matter) were used, and the DPPH radical scavenging activity of the insoluble powdered samples was measured by mixing insoluble matter with DPPH reagent (1.7 ml, 0.1 mM in methanol), and efficiently mixing after 0, 5, 15 and 25 min to facilitate the surface reaction between the insoluble material and the DPPH reagent (Serpen, Capuano, Fogliano, & Gökmen, 2007). The samples were then centrifuged and the absorbance of the clear supernatant was measured at 517 nm. For the *liposome model*, the liposomes were prepared of phosphatidylcholine to a final concentration of 0.8 wt.% (Huang & Frankel, 1997). The liposomes were incubated in sealed flasks in the dark at 37 °C in shaking water bath for 3 days in the presence of 2% flour sample (as powdered dry matter) of total liposome dispersion volume with 10 μM copper sulphate and 0.2 g/l of sodium azide (antimicrobial agent), after which 100 μl of sample was mixed with 5 ml methanol. Isolation of hydroperoxides was performed by centrifugation at 6000 rpm for 5 min, and conjugated diene hydroperoxides were measured spectrophotometrically at 234 nm from the clear supernatant. In both antioxidant models α-tocopherol and ferulic acid were used as positive controls (5 and 20 μM for DPPH and 10 μM for liposome model). *Lipoxygenase* (*LOX*) *activity* and spontaneous oxygen consumption of rye fractions were determined by following the consumption of oxygen after the addition of micellar linolic acid as described by Kaukovirta-Norja and Laakso (1993).