



Use of enzymes to elucidate the factors contributing to bitterness in rye flavour

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ABSTRACT

In spite of the health-beneficial character of whole grain rye its use may be limited because of bitter taste. The impact of non-volatile chemical compounds on the bitter taste of rye was analysed by the aid of enzymatic hydrolysis, releasing potentially flavour-active compounds from the rye matrix. Whole grain rye flour–water suspension was treated with hydrolytic enzymes, whereafter portions of the rye suspensions were baked into crackers, assessed for their sensory profile as well as solubilised hydrolysis products. Heat treatment reduced the perceived bitterness. The treatment with enzyme preparation with high protease activity increased the bitterness of rye and also wheat flour both as suspension and as crackers. Other enzymes tested (with high polygalacturonase, endo-glucanase, xylanase or amyloglucosidase activity) had no significant impact on the perceived bitterness. Thus, small molecular weight peptides were considered to be a significant contributor to the bitter note of rye.

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1. Introduction

The current high interest in cereal whole grain foods derives from epidemiological evidence suggesting their beneficial role in health maintenance in comparison with refined cereal foods. Epidemiological studies show that intake of whole grain cereal foods reduces the risk of type 2 diabetes (de Munter, Hu, Spiegelman, Franz, & van Dam, 2007) and cardiovascular disease (Jensen et al., 2004; Mellen et al., 2007). Health effects related to the consumption of whole grain foods are assumed to be derived from dietary fibre and phenolic compounds present mostly in the outer layers of cereal grain (Arranz, Saura-Calixto, Shaha, & Kroon, 2009; Okarter & Liu, 2010; Vitaglione, Napolitano, & Fogliano, 2008).

Dietary recommendations and the consumption of whole grain products do not meet due to several reasons. People do not necessarily understand the health benefits, they have difficulties in identifying whole grain foods (Adams & Engstrom, 2000; Lang & Jebb, 2003; Seal, Jones, & Whitney, 2006; Smith, Kuznesof, Richardson, & Seal, 2003, 2001; Smith, Smith, Richardson, & Seal, 2001), or they do not see the recommendations personally relevant (Lappalainen et al., 1998). Consumers tend to prefer refined wheat bread to whole wheat bread, if equivalent ingredients and procedures are used (Bakke & Vickers, 2007). Bakke and Vickers (2007) showed, however, that ingredient or processing modifications can improve liking of whole wheat bread to the level of refined bread.

Certain intrinsic flavours of whole grain are often not regarded attractive by consumers (Marquart et al., 2007). In particular flavour of whole grain rye is intensive and bitter, and may thus limit rye product applications. People vary widely in their sensitivity to bitter compounds, and several transduction mechanisms are suggested to exist (Delwiche et al., 2001). However, the bitter taste of rye is not equal with the cereal, rye-like flavour (Heiniö, Liukkonen, Katina, Myllymäki & Poutanen, 2003). Bitterness has been shown to be localised in the outer layers of the grain, mainly in the bran fraction (Heiniö, Liukkonen, Katina, Myllymäki & Poutanen, 2003). Volatile compounds in the air-space of a product have traditionally been explained to be a reason for flavour formation of cereal products (Kirchhoff & Schieberle, 2001). It is, however, obvious that non-volatile chemical compounds can bind to the taste receptors and influence taste perception, although their role on cereal flavour is not so well understood. The contribution of phenolic compounds and/or small peptides and amino acids and/or fatty acids on the bitterness of rye has been suggested (Hansen, 1995; Heiniö, Katina, et al., 2003; Heiniö et al., 2008; Liukkonen et al., 2003; Schieberle, 1996; Shewry & Bechtel, 2001).

We have previously studied the impact of various processing techniques on the flavour of rye (Heiniö, 2003; Heiniö, 2006 and Heiniö, Katina, et al., 2003; Heiniö, Liukkonen, Katina, Myllymäki & Poutanen, 2003). By milling fractionation the kernel can be divided into fragments with a distinct flavour profile (Heiniö, Liukkonen, Katina, Myllymäki & Poutanen, 2003). The endospermic fraction of grain has a very mild flavour, resembling the flavour of wheat, whereas the bitter, intense flavour and aftertaste are located in the bran fraction. On the other hand the shorts, with high bioactivity, have a cereal-like but not bitter flavour (Heiniö, Liukkonen, Katina, Myllymäki & Poutanen, 2003). Sourdough fermentation followed by extrusion

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has been shown to produce sour, intense flavour and aftertaste to rye (Heiniö, Katina, et al., 2003). During sourdough fermentation process proteolysis takes place, resulting in increased peptide and amino acid content (Gänzle et al., 2008; Loponen et al., 2009; Thiele et al., 2003). The proteolysis is caused by endogenous enzymes present in grain, by microbial enzymes from contamination of the grains and by enzymes secreted by sourdough bacteria.

Enzymes have effectively been used to improve the technological quality attributes of cereal products in general as reviewed by Tenkanen et al. (2000) and Rastall (2007), but their effect on the perceived flavour is not so well understood. In this work different types of commercial enzymes were used to modify the composition of rye flour, whereafter the perceived rye flavour was determined. Reasons for causes for rye bitterness were subsequently suggested.

2. Material and methods

2.1. Enzymes

The enzymes used were commercial multienzyme preparations containing several activities (Table 1). The key activities of the selected enzyme preparations were determined as described below: polygalacturonase activity was measured according to Bailey and Pessa (1989), endoglucanase activity according to IUPAC (1987), amyloglycosidase according to Bailey and Pessa (1989), endoxylanase according to Bailey et al. (1992), β -glucosidase and protease according to Bailey and Linko (1990), and lipase according to Lowry et al. (1951).

Activity profiling showed that the commercial enzyme preparations tested included several enzyme activities (Table 2). The activity profile of Pectinex BE 3-L and Corolase 7089 was narrower than that of other analysed preparations.

2.2. Flour

Medium coarse wholemeal rye flour (Paakari) used in the experiments was manufactured by Raisio Group, Finland. White rye flour (Mylly Matti) was manufactured by Helsingin Mylly Oy, Finland, and special white wheat flour (Sunnuntai) by Raisio Group, Finland.

2.3. Enzymatic treatments

The rye samples – with or without enzymatic treatments – were prepared either as rye flour–water suspensions or after a heating process as rye crackers. Rye flour–water suspensions (40 g rye flour + 60 g water) were incubated with enzymes for 2 h at +40 °C by mixing occasionally with a spoon. The reference sample without enzyme addition was incubated accordingly. Small rye crackers (approximately 3 cm in diameter) were prepared of the rye flour–water suspension by using two teaspoons, and the surface of the crackers was perforated with a fork. The crackers were baked for 15 min (for 2.5 min using steam and the rest 12.5 min with a convection mode) at 175 °C on a baking paper in a convection oven. The impact of endogenous enzymes on rye flavour was elucidated by analysing both non-incubated and incubated (2 h +40 °C) rye flour–water suspensions.

The enzyme dosages used in the screening trials are presented in Table 1. In further experiments the enzymes were dosed according to the selected activity as indicated in Table 2: 10 and 100 nkat activity/g of rye flour, except protease (Corolase 7089) was dosed 1 and 10 nkat/g rye flour.

The impact of protease preparation (Corolase 7089) (5 nkat activity/g flour) was further elucidated using wholemeal rye, white rye and white wheat flours with an attempt to verify the possible influence of released peptides on the perceived bitterness. The corresponding flours were analysed also without enzymatic treatment. In sensory evaluation both flour–water suspensions and baked crackers was studied.

2.4. Sensory evaluation

All sensory work was carried out at the sensory laboratory of VTT, which fulfils the requirements of the ISO standards (ISO, 1985, 1988). The sensory panel consisted of ten trained assessors with proven skills. All assessors of the internal sensory panel have passed the basic taste test, the odour test and the colour vision test, and their evaluation ability is routinely checked using individual control cards for each assessor. The panel was particularly familiarized with the sensory descriptors and the attribute intensities of rye and processed rye in several pre-sessions prior to the evaluations with real samples,

Table 1
Commercial enzyme preparations used in the preliminary trials, activities and used dosages.

Commercial name	Producer	Reported main activities (main function examined in this study presented in bold)	Main activity	Enzyme dosage nkat/g flour
Pectinex BE 3-L	Novozymes	Polygalacturonase , pectin lyase, pectin methylesterase	90,000 nkat PG/ml	1000
Econase CE	AB Enzymes	Xylanase, endo-glucanase and β -glucosidase	16,600 nkat EG/ml	200
Veron CLX	AB Enzymes	α-Amylase , transglutaminase, xylanase	4550 U AMY/g	50
Pentopan mono PG	Novozymes	Polygalacturonase, xylanase , endo-glucanase, amylase, phenolic acid esterase	361,000 nkat XYL/g	1000
Novozym 188	Novozymes	β-Glucosidase , α -galactosidase, mannanase	5300 nkat B-GL/ml	200
Corolase 7089	AB Enzymes	Endo-peptidase	177 nkat PRO/ml	10
Corolase LAP	AB Enzymes	Endo-peptidase	350 LAP/ml ²	3.5 LAP/g
Lipopan 50 BG	Novozymes	1,3-Specific lipase	505,100 nkat LIP/g	1000
Biobake Fresh XL	Quest Int.	Amyloglucosidase	8500 nkat/g	100

nkat: nanokatal. 1 nanokatal is defined as the amount of enzyme required to raise the rate of reaction by 1 nmol/s under defined assay conditions.

PG, polygalacturonase; EG, endo-glucanase; AMY, α -amylase; XYL, xylanase; B-GL, β -glucosidase; PRO, endo-peptidase activity on azocasein; LAP, Leucine aminopeptidase activity; LIP, lipase as measured as Kilo Lipase Units (KLU).

Table 2
Enzymatic activities in the commercial enzyme mixtures used in the treatments of the rye–water suspensions, where the main activity of the enzyme 100 or 10 nkat/ml (shown as bold).

Enzyme	Endo-glucanase nkat/ml	Polygalacturonase nkat/ml	Xylanase nkat/ml	Mannanase nkat/ml	Protease nkat/ml	Amyloglucosidase nkat/ml	β -Glucosidase nkat/ml
Pectinex BE 3-L	1	100	4	2	0	2	0
Econase CE	100	19	294	14	0	0	3
Novozym 188	5	210	72	36	0	2287	100
Corolase 7089	0	6	4	2	10	9	0
Biobake Fresh XL	0	476	41	35	0	100	0

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