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Stability of B-complex vitamins and dietary fiber during rye sourdough bread production

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The stability of vitamers: thiamine, riboflavin, nicotinic acid, nicotinamide, pantothenic acid, pyridoxine and pyridoxal, as well as soluble and insoluble dietary fiber was studied in a rye sourdough bread process. The vitamer concentrations were measured in raw materials (rye flours, white and red rye malt, yeast) and the rye sourdough breads made from them by means of LC–MS and stable isotope dilution assay. The content of dietary fiber was determined using a standard enzymatic-gravimetric method. During baking, the concentration of vitamins decreased by 20–45% in the case of thiamine, 25–50% in the case of nicotinic acid, 45–65% in the case of pyridoxal in both breads, 50% in the case of riboflavin and 15% in the case of pyridoxine only in fine rye bread. In contrast, the content of nicotinamide increased during processing by ten fold, presumably due to microbial activity during sourdough fermentation. The ratio of soluble to insoluble dietary fiber increased during rye sourdough processing.

1. Introduction

Rye sourdough bread is a traditional and popular bread in North–West Russia, the Baltic states, Finland, Denmark, and the North of Germany. Rye sourdough bread is industrially produced by mixing sourdough with flour, followed by fermentation with yeast (leavening), and baking. Rye bread is a recommended part of the diet because it is a good source of various bioactive substances, including B-complex vitamins and dietary fiber.

In industrial product labeling practice, the calculation of the content and composition of vitamins and fiber is based on the composition of their ingredients. However, this may result in incorrect estimations, mostly due to variations in the vitamins and fiber content of the particular ingredients used, but also due to chemical changes that occur during processing.

Dietary fiber is an indigestible complex of carbohydrates and lignin. It is an important functional component, the content of which can change during rye bread processing. Insoluble dietary fiber compounds in rye include cellulose (1–3 %), lignin (1–2 %) (Liukkonen et al., 2006) and the insoluble part of arabinoxylans. Rye grain contains 8–12 % of arabinoxylans, fructan (4.5–6.4%) and β-glucans (1–3%) (Hansen et al., 2003; Karppinen et al., 2003; Liukkonen et al., 2006). The soluble fibers are fructo-oligosacchrides 1-kestose (0.6%), 1,1-kestotetraose (0.3%), and 1,1,1-kestopentaose (0.3%) (Karppinen et al., 2003). Amount of water-extratable β-glucans is 1.2% in rye (Aura et al., 2007).

Enzymatic as well as thermal treatment can modify the ratio between soluble and insoluble fibers during bread making (Elleuch et al., 2011). During flour hydration, several intrinsic hydrolytic enzymes of cereals (α-amylase, β-xylosidase, α-L-arabinofuranosidase, cinnamoyl esterases, D-xylosidase, β-glucanases and endo-D-xylanase) are activated (Boskov Hansen et al., 2002). Also the enzymes with β-glycolytic activity from lactic acid bacteria (Axelsson, 2007) and yeast (Hauf et al., 2000) can affect the fiber concentration during rye bread processing. The most commonly used methods for the determination of dietary fiber in cereals are enzymatic-gravimetric methods (Lee et al., 1992).

The term *vitamin* refers to a number of *vitamer* compounds with the same biological activity as the respective vitamin (Table 1). Unfortunately, the complete list of vitamers with respective vitamin activity for mammals is still not clear. Several vitamins during food processing, storage and digestion are derived from cofactors as the result of enzymatic activities in both the food matrix and the digestive tract. For example, 2–10% of the vitamin B1 pool in cereals is present as its phosphorylated form thiamine diphosphate (TDP) (Buchholz et al., 2011), which is hydrolyzed by different phosphatases in the human gastrointestinal tract into

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Table 1
Vitamins and corresponding vitamers.

Vitamin	Vitamers
B1, thiamine	Thiamine
	Thiamine monophosphate (TMP)
	Thiamine diphosphate (TDP)
	Thiamine triphosphate (TTP)
	2-(1-hydroxyethyl)thiamin (HET)
B2, riboflavin	Riboflavin
	Flavin mononucleotide (FMN)
	Flavin adenine dinucleotide (FAD)
B3, niacin	Nicotinic acid
	Nicotinamide
	Nicotinamide adenine dinucleotide (NAD)
	Nicotinamide adenine dinucleotide phosphate (NADP)
	Nicotinic acid adenine dinucleotide (NAAD)
	Nicotinic acid adenine dinucleotide phosphate (NAADP)
	N-ribosyl nicotinamide
	N-ribosyl nicotinic acid
B5, panthothenic acid	Pantothenic acid
	Coenzyme A (CoA)
	Phosphopantothenic acid
	Pantetheine
	Phosphopantetheine
B6, pyridoxine	Pyridoxine
	Pyridoxal
	Pyridoxamine
	Pyridoxine phosphate (PNP)
	Pyridoxal phosphate (PLP)
	Pyridoxamine phosphate (PMP)
	5′-O-β-D-glucopyranosyl pyridoxine

thiamine (Rindi and Laforenza, 2000). Similarly, the phosphorylated forms of riboflavin and vitamin B6 can also be found in nature (Cataldi et al., 2002; Roth-Maier et al., 2002).

In practice, the content of B-complex vitamins is expressed as the concentrations of their most simple commercially available chemical forms (e.g. B1 = thiamine chloride hydrochloride, B2 = riboflavin, niacin = nicotinic acid + nicotinamide, expressed as nicotinic acid, B5 = calcium pantothenate, B6 = pyridoxine + pyridoxal + pyridoxamine, expressed as pyridoxine hydrochloride) which often serve as a starting point for the synthesis of respective cofactor(s) in cells. Thus, in most of the food databases, the B-complex vitamin contents (μg per 100 g of food) are expressed as thiamine, riboflavin, niacin, pantothenic acid and pyridoxine hydrochloride equivalents.

Information regarding the stability of B-complex vitamers during the course of rye sourdough bread-making processes is scarce. Thiamine is relatively stable at pH 2.0–4.0, which is a common pH range of the sourdough. In slightly acidic solutions it is stable up to the boiling point. However, during baking, 20–50 % losses of thiamine have been reported (Martinez-Villaluenga et al., 2009). Similarly, riboflavin is relatively heat-stable (Batifoulier et al., 2005). It has been reported that whole wheat bread made with yeast results in 30% enrichment in riboflavin due to the contribution of endogenous yeast riboflavin, while sourdough lactic acid fermentation without yeast did not result in any increase in the bread's riboflavin content (Batifoulier et al., 2005).

Nicotinamide and nicotinic acid are relatively stable during baking (Ottaway, 2002). A small part of chemically bound nicotinic acid (i.e. NAD) can be hydrolyzed by gastric juice and is thus converted into bioavailable forms (Wall and Carpenter, 1988). Pantothenic acid has good stability during most food processing operations, including baking (Ottaway, 2002). Pyridoxine, pyridoxal and pyridoxamine are relatively heat-stable under acidic conditions and very heat-labile under alkaline conditions (Leklem, 2001). The baking of bread can induce losses of up to 17% for vitamin B6 (Ottaway, 2002).

The current study was carried out to analyze the stability of soluble and insoluble dietary fiber and B-complex vitamers during rye sourdough bread making. To determine vitamer concentrations we used a high throughput LC–MS and stable isotope dilution assay.

2. Materials and methods

2.1. Materials and reagents

Whole grain rye flour R1800, dark rye flour R1370, fine rye flour R705, wheat flour W700, inactive (red) rye malt, active (white) rye malt and steel cut rye grains were obtained from Tartu Grain Mill Ltd. (Tartu, Estonia). The same lots of flour were used for the production of all bread recipes. The commercial rye bread containing dark rye flour (55%), active rye malt (1%) and inactive rye malt (1%), yeast (0.9%), and fine rye bread containing fine rye flour (42%), wheat flour (10%), dark rye flour (7%), active rye malt (1%), and yeast (1%) were obtained from a local bakery.

Dark rye flour (R1370) used for incubation and fermentation experiments was sterilized by γ-irradiation at 10 kGy using a dosimetric system GEX WinDose (Centennial, CO, USA). Bacterial strain *Lactobacillus panis* N915 was isolated from the rye sourdough (Mihhalevski et al., 2011). Fresh baker's yeast was kindly provided by AS Salutaguse Pärmitehas (Kohila vald, Estonia).

Certified reference material BCR 121 (wholemeal flour) was obtained from the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium). The certified values of B-complex vitamers with uncertainties (μg/100 g dry matter) for BCR 121 were as follows: 364 ± 31 for vitamin B1 (expressed as thiamine equivalents) and 377 ± 84 for B6 (expressed as pyridoxine equivalents).

Acid washed Celite was obtained from Megazyme International Ireland Ltd. (Bray, Ireland). Acetone and acetonitrile (both HPLC grade) were obtained from Rathburn Chemicals Ltd. (Walkerburn, Scotland, UK), MES (2-(N-morpholino) ethanesulfonic acid), ammonium formate (puriss.p.a., for HPLC), HCl (37% puriss.p.a.) and TRIS (tris(hydroxymethyl) aminomethane) were obtained from Sigma–Aldrich Inc (St.Louis, MO, USA), NaOH was obtained from Lach-Ner (Neratovice, Czech Republic) and Sigma–Aldrich. High purity water was produced by a Millipore water purification system (Millipore S.A.S., Malsheim, France).

Nicotinamide (99.9%), nicotinic acid (99%), thiamine chloride hydrochloride (99%), pyridoxine-HCl (99.9%), pyridoxal-HCl (99%), Ca-pantothenate (99%) and riboflavin (99%), used as external standards were obtained from Sigma–Aldrich. The stable isotope labeled internal standards nicotinamide – [D_4, 98%], nicotinic acid – [D_4, 98%], thiamine-Cl – [$^{13}C_3$, 99%], pyridoxine-HCl – [$^{13}C_4$, 99%], were obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Ca-pantothenate – [$^{13}C_3$,^{15}N, 97.1%], riboflavin – [$^{13}C_4$,$^{15}N_2$, 97.9%] and pyridoxal HCl – [D_3, 99%] were from Isosciences, LLC (King of Prussia, PA, USA).

α-amylase from *Aspergillus oryzae* (crude, Cat. No. A6211), β-glucosidase from almonds (crude, Cat. No. G0395), and potato acid phosphatase (Cat. No. P1146) were obtained from Sigma–Aldrich.

Thiamine monophosphate chloride dehydrate (99%), thiamine pyrophosphate chloride (≥97%), riboflavin 5′-monophosphate sodium salt dehydrate (75.9%), flavin adenine dinucleotide disodium salt hydrate (97%), β-nicotinamide adenine dinucleotide hydrate (98%), β-nicotinamide adenine dinucleotide reduced disodium salt hydrate (99%) and coenzyme A hydrate (99%), were obtained from Sigma–Aldrich.