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Se-enriched sprouted seeds as functional additives in sourdough fermentation

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ABSTRACT

Sprouted seeds possess potential to stimulate the growth and acidifying activity of lactic acid bacteria. Simultaneous enrichment of the sprouted seeds in deficient selenium may create a multifunctional additive to sourdough fermentation with consequent supplementation of human diet. Bread being a staple diet seems to be a perfect carrier of this micronutrient.

In the study germinating seeds revealed high ability to accumulate selenium from watering solution, up to 188 mg Se/kg of dried sprouts in the case of lentil. The process of selenium accumulation strongly depended on the plant species resulting in total Se content in soy and lentil biomass twice as high as in rye or wheat sprouts. A high correlation ($R^2 = 0.97$) between selenium concentration in water and Se accumulation in biomass was observed. Raising of pH from 7.0 to 8.0 increased 2.5 times selenium uptake.

The addition of Se-enriched plant biomass had stimulating effect on the sourdough fermentation process allowing to shorten it by 8–16 h, depending on the dose and temperature. Se fortified rye sprouts used in amounts 2.5 g/100 g of flour did not deteriorate sensory quality of bread and raised five times selenium content in it.

1. Introduction

Sourdough fermentation is a traditional process in bread production especially popular in Central and Eastern Europe (Diowksz & Ambroziak, 2006). However nowadays in many countries its application is often limited to artisan bakeries mainly due to a time-consuming character of the production process. Its adaptation to modern technology requires effective methods to enhance fermentation.

Sprouted seeds are well known sources of enzymes and nutritive compounds with potential to stimulate the growth and metabolism of microorganisms. Although this phenomenon is commonly used for barley applied in a form of malt in beer production, the same effect is observed in the case of other seeds like wheat, oat, rye, maize, rice, sorghum, millet, buckwheat and quinoa (Kaukovirta-Norja, Wilhelmson, & Poutanen, 2004; Kreisz et al., 2005; Zarnkow, Kessler, Burberg, Kreisz, & Back, 2005). Also lactic acid bacteria efficiently use nutrients originating from malted cereals revealing elevated acidifying activity (Lowe & Arendt, 2004).

Enrichment of the seeds in deficient micronutrients during sprouting may create a multifunctional additive to sourdough fermentation and consequently supplement the human diet.

Selenium as the essential trace element has attracted attention for the last decades due to many evidences indicating that Se deficiency states may be related to a variety of degenerative diseases (Arthur, McKenzie, & Beckett, 2003; Combs, 2001; Neve, 1991; Rayman, 2000). Dietary daily selenium intakes in many European countries are currently much lower than recommended ones (Combs, 2001; Rayman, 2004; Stabnikova, Ivanov, Larionova, Stabnikov, Bryszewska, & Lewis, 2008; Wasowicz, Gromadzinska, Rydzynski, & Tomczak, 2003). In these regions to improve Se status in population to a proper level selenium supplementation should be implemented.

The use of Se-fortified fertilizers is however considered as too wasteful to be applied to large areas. Only a small proportion of Se, less than 20%, is taken up by plant whereas much of the reminder is lost for future utilisation. Thus direct addition of selenium compounds to food is much more advisable (Haug, Graham, Christophersen, & Lyons, 2007).

Bread being a staple diet seems to be a perfect carrier of this micronutrient (Bryszewska et al., 2005; Diowksz, Peczkowska, Wlodarczyk, & Ambroziak, 2000; Stabnikova et al., 2008). Additionally, selenium introduced with Se-enriched sprouted seeds

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would reveal much higher bioavailability than inorganic supplements (Fairweather-Tait, 1997; Schrauzer, 2000). During sprouting inorganic selenium undergoes biotransformation into organic forms incorporated hereafter in the newly synthesized protein. As speciation analysis confirmed (Bryszewska et al., 2005; Diowksz et al., 2000) sprouting of seeds in the presence of selenite results in the raise of selenomethionine content in the plant biomass.

The aim of the study was to assess the effectiveness of selenium uptake by germinating seeds and to determine influence of Se-enriched sprouting seeds on sourdough fermentation.

2. Materials and methods

2.1. Preparation of Se-enriched sprouted seeds

Rye (*Secale cereale*), wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), maize (*Zea mays*), soy (*Glycine max*) and lentil (*Lens esculenta*) seeds were used in the preparation of Se-enriched biomass. Before germination overnight steeping process was performed with Se containing water solution (SeO2, Baker, Netherlands) in concentrations 10 or 20 mg Se/L. Germination was performed on grooved trays watered with Se solution twice a day. The process was carried out for up to 6 days at room temperature, pH 7.0 or 8.0, in the normal day–night cycle or in darkness. The resulted sprouted seeds were harvested, washed with distilled water, dried at 45 °C and ground. The moisture content in dried and ground sprouts amounted 4.8 g/100 g.

2.2. Se-supplemented sourdough fermentation and bread making

Se-enriched 5-days germinated rye seeds in dried form were used as additives in sourdough fermentation. The plant biomass was added to rye sourdoughs prepared with the yield of 250 (flour and water in the ratio of 1:1.5) according to the one-stage fermentation pattern in amounts of 2.5, 5 or 7.5 g/100 g of flour. The starter culture used in the amounts of 2 mL/100 g of flour was composed of lactic acid bacteria of *Lactobacillus* species (*Lb. plantarum* AD-98, *Lb. sanfranciscensis* MW-94, *Lb. brevis* MS-99) and yeast *Saccharomyces cerevisiae* K1 previously cultivated in Difco Lactobacilli MRS Broth at 30 °C for 24 h. All strains were derived from Centre of Industrial Microorganisms Collection (LOCK 105). Fermentation was performed in temperature range 20–30 °C for 24 h. The progress of fermentation was monitored by titratable acidity measurements.

Dough for mixed wheat-rye bread (rye flour to wheat flour ratio 30:70) was prepared by addition of wheat flour and tap water in amounts required to achieve dough yield 160. Salt (1.5 g/100 g of flour) and baker's yeast (2 g/100 g of flour) were also added. After first proofing at 30 °C for 40 min the dough was divided into 450 g pieces and placed in pans. Second proofing was performed at 30 °C for 30 min. Bread was baked at 220 °C for the first 10 min and at 180 °C for the rest 30 min with low pressure steam introduced into the oven at the beginning of baking process (Polin, Verona, Italy). Control bread was produced without Se-additives. Three independent batches (two loaves per batch) were performed.

Scoring method according to Polish Standards (Polish Standards, 1996) was used for the estimation of bread quality. A trained sensory panel (2 females and 3 males, aged 24–53) evaluated quality of the breads. Bread inspections included sensory analysis and instrumental assessment of volume, crumb moisture and acidity. Sensory analysis involved assessment of appearance, crust, crumb and flavour. Each parameter was awarded as either very good, good, satisfactory or poor. Each description corresponded to a score (Table 1). Breads were qualified into a proper quality level (Table 2).

Table 1
Scoring for bread quality parameters.

Quality parameter	Score			
	Very good	Good	Satisfactory	Poor
Appearance	5	4	0	−35
Crust				
Colour	3	2	0	−35
Thickness	4	3	0	−35
Other features	4	3	0	−35
Crumb				
Resilience	4	3	0	−35
Porosity	3	2	0	−35
Other features	3	2	0	−35
Flavour	6	5	0	−35
Physico-chemical parameters				
Volume	3	2	1	−35
Moisture	2	–	–	−35
Acidity	3	–	–	−35

2.3. Selenium determination

Selenium content in the samples after acid mineralization was determined by the fluorimetric method of Watkinson (Watkinson, 1966) with 2,3-diaminonaphthalene (Luminescence Spectrometer LS 50B, Perkin Elmer, UK). Bovine Liver CRM no. 185 (Community Bureau of Reference, Brussels, Belgium) served as Se level reference.

2.4. Statistical analysis

The results represent the means and standard deviations (SD) of triplicate determinations. Statistical significance of the data was determined by Student's *t*-test. A p value below 0.05 was considered statistically significant.

3. Results and discussion

3.1. Seed germination

Screening tests for different plant seeds used in the study exhibited their diverse ability to accumulate selenium from watering solution (Fig. 1). Only in the case of rye and wheat germinated in the presence of 10 mg Se/L of water the differences in the final concentration of selenium in plant biomass were not statistically significantly different ($p < 0.05$). These observations are in conformance with general mechanism occurring in higher plants indicating considerable differences in their physiological response to selenium (Terry, Zayed, de Souza, & Tarun, 2000). In the present study among the examined plants the highest accumulation of Se was observed for soy and lentil amounting 77.1 mg Se/kg of soy dried sprouts and 90.9 mg Se/kg of lentil dried sprouts, watered during germination with Se solution in the concentration of 10 mg Se/L, or 132.0 and 188.0 mg Se/kg, respectively, at twice higher concentration of watering solution. The total Se content in rye and wheat sprouts was twice lower ($p < 0.05$). It can be explained by high content of proteins in legumes (25% in lentil and

Table 2
Quality levels in bread assessment method.

Quality level	Total score
I	40 ÷ 36
II	35 ÷ 31
III	30 ÷ 26
IV	25 ÷ 8
Disqualification	<8