Content and in vitro bioavailability of selected B vitamins and *myo*-inositol in spelt wheat (*Triticum spelta* L.) subjected to solid-state fermentation

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Summary

This study examined the effects of tempe-type fermentation of spelt and green spelt grains with *Rhizopus oligosporus* on the content and in vitro bioavailability of B-group vitamins riboflavin and thiamine, as well as *myo*-inositol, a bioactive compound. The fungal fermentation resulted in approximately 2.5-fold increase in the riboflavin content in spelt. The bioavailability of B_2 vitamin was high (65–86 %) and did not change in result of the treatments applied (boiling followed by fermentation). The content of thiamine was increased in tempe-type products made from spelt (by 6 %) and green spelt (by 31 %), as compared to boiled grains, which partially compensated the loss (15–36 %) of B_1 vitamin after hydrothermal treatment. The fermentation was beneficial for the pool of bioavailable *myo*-inositol, which was higher by 37 % in the case of spelt and by 47 % in green spelt than that of the respective boiled grains. Tempe contained 100 mg (spelt) and 142 mg (green spelt) of dialysable *myo*-inositol per kilogram of dry product.

Keywords

Triticum spelta; solid state fermentation; B-group vitamins; *myo*-inositol; in vitro bioavailability

Cereals are traditionally considered as staple food but their consumption has decreased in recent years due to changes in the lifestyle. New cereal products developed by the industry provide the human body with nutritional and functional benefits, including micronutrients such as minerals and vitamins, as well as other bioactive substances. Thiamine and riboflavin are two vitamins involved in key metabolic processes in the human body. Their active phosphorylated coenzymatic forms (flavin adenine dinucleotide, flavin mononucleotide and thiamine pyrophosphate) participate i.a. in gluconeogenesis, glycolysis, the Krebs cycle, neurotransmission, gene replication and fetal tissue development [1].

Technological processing to which cereal grains are subjected, e.g. grinding, hydrothermal treatment or exposure to light radiation, can partially degrade B-group vitamins in the range from 25 % (thiamine) to even 50 % (riboflavin) [2, 3]. Therefore, many industrial cereal products are enriched with the said compounds [4, 5]. In this context it should be mentioned that a significant part of the population suffers from riboflavin or thiamine deficiency and so enriching food products in these nutrients is aimed at reducing the risk of the incidence of Wernicke-Korsakoff syndrome [6, 7]. Alternatively, the pool of the aforementioned compounds in food products can be increased by means of microbial processing, such as solid state fermentation (SSF). This kind of biotreatment can be applied to pseudo-cereals and organic cultivars of cereals, such as spelt, whose popularity has been recently restored [8–10].

Myo-inositol is a hexahydroxylated cyclohexane derivative found in varying amounts (from 0.03 $mmol \cdot l^{-1}$ in blood plasma to 3 $mmol \cdot l^{-1}$ in brain tissue) in physiological fluids and metabolically active organs, such as the brain, liver or kidney [11]. The compound is considered an immunostimulant, an important drug in the treatment of psychiatric disorders, retinopathy and many other diseases [12–14]. It is formed as a result of the final stage of conversion of phytates, the main phosphorus reservoir present in significant amounts (0.5–2.0 %) in cereal seeds, being regarded as

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an antinutrient due to its ability to chelate divalent metal ions (Mg^{2+}, Ca^{2+}, Zn^{2+}, Fe^{2+}) [15]. As shown by the results of a pioneer research, *myo*-inositol can be generated as a result of simultaneous catalytic action of phytases and phosphatases, which are enzymes present in plant material and microorganisms [16].

The main objective of this paper was to assess the influence of tempe-type processing, i. e. boiling and subsequent fermentation with *Rhizopus oligosporus*, on vitamin B_1 (thiamine) and B_2 (riboflavin) content in spelt wheat (*Triticum spelta* L.), and their in vitro bioavailability. Another aim of the study was to attempt an estimation of the in vitro bioavailability of free *myo*-inositol from the material. The potential of this bioactive compound, until recently considered as B_8 vitamin, is not yet fully explored. It can be regarded as an added value of cereal products developed on the basis of processed, fermented whole grain spelt, which fits perfectly the definition of functional food.

MATERIAL AND METHODS

Spelt (mature grains) and green spelt (immature grains) were purchased from a "healthy food" store in Kraków, Poland. *Rhizopus oligosporus* ATCC 64063 was grown on potato dextrose agar (PDA, Sigma-Aldrich, St. Louis, Missouri, USA) at 24 °C for 12 days. The spores were harvested with a sterile saline solution (8 g·l^{-1}) supplemented with peptone (0.01 g·l^{-1}) and Tween 80 (0.1 ml·l^{-1}). The spore suspension was filtered through a nylon filter (pore size 11 μm; Merck-Millipore, Billerica, Massachusetts, USA) and the suspension density was determined by counting in a Thoma chamber.

Cooking procedure

The grains were boiled in tap water acidified with lactic acid to pH 4.5–5.0 (to obtain proper conditions for fungal growth) for 20 min (green spelt) or for 25 min (mature spelt).

Fermentation procedure

Drained grains were dried on a surface, cooled (< 30 °C) and aseptically mixed with *R. oligosporus* spore inoculum (10^4 spores per gram of dry grains). Next, the material was packed in sterile Petri dishes (4 replications for each spelt kind) and incubated at 31 °C for 30 h. The fungal growth was stopped by steaming the grains for 10 min. The cooked and fermented grains were lyophilized and stored for a maximum of 1 week at 3 °C until analysed.

Analysis of vitamin B_1 and vitamin B_2

Detection of B_1 and B_2 vitamins was done as described by Starzyńska-Janiszewska et al. [8]. The separation of riboflavin and thiamine as thiochrome was done by reversed-phase high-performance liquid chromatography (HPLC) using Luna C18 column (250 mm × 4 mm, particle size 5 μm; Phenomenex, Torrance, California, USA) isocratically with a mobile phase that consisted of methanol and 0.05 mol·l^{-1} sodium acetate (30 : 70, v/v), at a flow rate of 1 ml·min^{-1}. The fluorometric detector was set at excitation wavelengths of 366 nm and 422 nm, and emission wavelengths of 435 nm and 522 nm for vitamin B_1 and vitamin B_2, respectively. Conversion of thiamine to thiochrome was done by post-column modification with an oxidizing reagent 0.1% potassium hexacyanoferrate(III) in 12% NaOH pumped by a peristaltic pump at a flow rate of 0.2 ml·min^{-1}.

Determination of in vitro bioavailability

The in vitro bioavailability of riboflavin, thiamine and *myo*-inositol was estimated according to the method described by Żyła et al. [17], which was modified in order to simulate human stomach and small intestine conditions. A 0.5 g sample of the material was incubated at 37 °C and pH 2 for 2 h with 1.7 mg of pepsin (declared activity 4 750 U·mg^{-1}; Sigma-Aldrich) dissolved in 0.1 mol·l^{-1} HCl. Next, 2.5 mg of pancreatin (from porcine pancreas, 8 x USP specifications; Sigma-Aldrich), dissolved in 0.1 mol·l^{-1} $NaHCO_3$, was added. The mixture was transferred to dialysis tubes with a cellulose membrane (molecular weight cut off 12 000 Da, Sigma-Aldrich) and incubated for 4 h at 37 °C in flasks containing 50 ml of imidazole buffer (pH 7.0). The dialysates obtained were used for HPLC analysis.

The term "in vitro bioavailability" was defined as the ratio between the amount of compounds in the dialysate (in this case *myo*-inositol, vitamin B_1 or vitamin B_2), which passed through the pores of the dialysis membrane and was found in the buffer solution after simulated in vitro digestion, and their amount in the material, being expressed in weight percent.

HPLC analysis

Proper amounts of homogenized samples (150 mg) were subjected to acid hydrolysis with 2 ml of 1 mol·l^{-1} HCl in Duran glass tubes (16 mm diameter,·160 mm length; Schott, Mainz, Germany) for 48 h at 123 °C. After cooling the sample, the pH value was adjusted to 4.8–5.0 and the sample was subjected to HPLC analysis according to Duliński et al. [18]. The same procedure was applied to samples obtained from the in vitro gastrointestinal simulation to provide data on dialysable *myo*-inositol.

Statistical analysis

Experimental data were subjected to one-way analysis of variance (ANOVA) and expressed as a mean ± standard deviation. Tukey's post-hoc test was applied (at $p \leq 0.05$) to determine statistically significant differences. Data were processed using the software Statistica for Windows, ver. 13.1 (Statsoft, Tulsa, Oklahoma, USA).

RESULTS AND DISCUSSION

Thiamine

The content of thiamine in the grains of raw spelt (3.46 mg·kg^{-1}) and green spelt (3.08 mg·kg^{-1}) (Fig. 1) was higher than the values of 1.47–1.99 mg·kg^{-1} determined previously for common wheat (*Triticum aestivum*) [19] but lower than data available for selected spelt varieties Balmeg and Poeme (5.83–6.18 mg·kg^{-1}) [20].

Hydrothermal treatment resulted in the expected significant decrease in the thiamine content in grains in the range of 15–36 % (Fig. 1). Subsequent fermentation with *R. oligosporus* resulted in a 33% increase in B_1 vitamin content in green spelt (to 2.94 mg·kg^{-1}). In the case of mature grains, statistically significant differences were also noted between boiled seeds (2.62 mg·kg^{-1}) and tempe (2.78 mg·kg^{-1}). A positive effect of *R. oligosporus* ATCC 61063 activity on the thiamine content was previously observed for fair buckwheat groats [8]. However, tempe-type fermentation with *R. oligosporus* does not always result in the enrichment of the plant material in B_1 vitamin. An important factor is the fermentation substrate, as shown in the case of tempe-type fermentation of coloured and white quinoa [21] as well as raw and roasted buckwheat groats [22]. As the content of thiamine in the product is a result of microbial synthesis and its utilization by a microorganism, different effects of fermentation were observed. For example, a significant decrease in thiamine content was observed after a 24 h fermentation of bambara nut (*Vigna subterranea* (L.) Verdc.) flour with *R. oligosporus* [23]. A different tendency was found in the case of bacterial fermentation of tarhana (traditional Turkish fermented wheat-flour-yoghurt mixture), which did not change the content of the said vitamin in the material [24].

Riboflavin

The content of riboflavin in raw grains of green spelt was considerably higher (1.64 mg·kg^{-1}) than values reported for common wheat (0.67–0.82 mg·kg^{-1}) and spelt cultivars Balmeg and Poeme (0.77–0.80 mg·kg^{-1}) [19, 20]. However, raw grains of mature spelt were characterized by a relatively low content of vitamin B_2 (0.5 mg·kg^{-1}). Hydrothermal treatment resulted in a significant decrease of riboflavin content in the material, by 32 % on average (Fig. 1).

An important finding was a nearly 2.5-fold increase in the riboflavin content as an effect of the solid-state fermentation of mature spelt grains (to 1.07 mg·kg^{-1}). This effect also related to green spelt tempe, which contained by 67% higher content of vitamin B_2 than the respective boiled grains (Fig. 1). This observation confirmed previous reports concerning solid-state fermentation with *Rhizopus* sp., in which a 1.5–7.5-fold enrichment in riboflavin was noted, depending on the substrate (grass pea, pseudocereals) [21, 25]. In a different study, the ability to synthesize, among others, vitamins B_2, B_6 and nicotinamide during the tempe-type fermentation of soya seeds was observed, and *R. oligosporus* strains were indicated as the best producers of these vitamins among several fungal and bacterial strains tested [26]. The attempts to select *Lactobacillus fermentum* strains for application in dough fermentation in order to obtain riboflavin-enriched wheat bread resulted in a high increase of the vitamin content by 3.30–6.67 mg·kg^{-1} of the product [27]. At the same time, data presented in this paper are consistent with the results of our previous study that concerned buckwheat groats fermented with the same *R. oligosporus* strain, in which we reported enrichment by 0.74 mg·kg^{-1} [8].

In vitro bioavailability of vitamin B_2 deter-

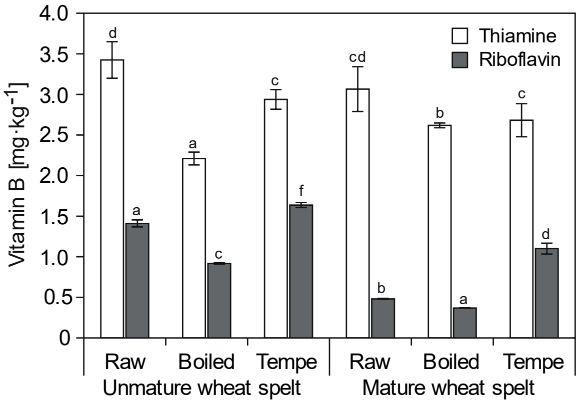

Fig. 1. Thiamine and riboflavin contents in spelt wheat (*Triticum spelta* L.) grains fermented with *Rhizopus oligosporus*.

Means ± standard deviation are presented. Mean values denoted by different letters differ significantly at $p \leq 0.05$.

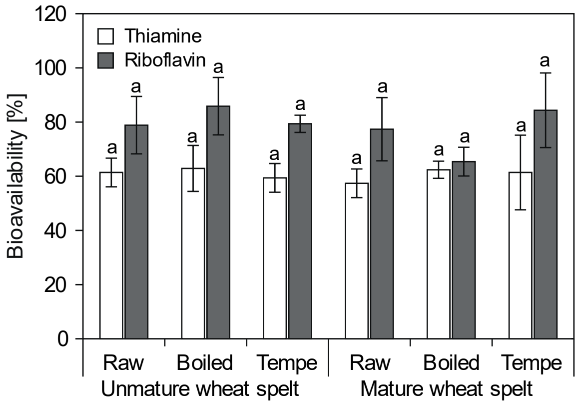

Fig. 2. In vitro bioavailability of thiamine and riboflavin from spelt wheat (*Triticum spelta* L.) grains fermented with *Rhizopus oligosporus*.

Means ± standard deviation are presented. Mean values denoted by different letters differ significantly at $p \leq 0.05$.

mined for spelt grains (65–86 %; Fig. 2) was higher than data presented by Kurek et al. [28] for wheat flour bread (47–50 %). In the case of vitamin B_1, the average in vitro bioavailability was similar to the range reported elsewhere for cereal materials (67 % for wheat bran) [29]. The applied treatments, namely boiling followed by fermentation, did not significantly influence the in vitro bioavailability of vitamins in spelt or green spelt (Fig. 2). However, it is worth emphasizing that the obtained spelt tempe products were characterized by a considerably higher riboflavin content, together with comparable in vitro bioavailability of this component, as compared to boiled grains (Fig. 1, 2). The high bioavailability of vitamin B_2, estimated by the in vitro digestion model, is an unquestionable advantage of boiled and fermented spelt and green spelt grains in the context of the deficiencies of this component caused by the processing of cereal products.

Myo-inositol

The determination of total *myo*-inositol includes all forms of *myo*-inositol deposited in phospholipids, cell membranes, phytates and other compounds potentially present in cells [30]. In the case of this parameter, no statistically significant differences were found between the immature and mature spelt grains or after the applied treatments (Tab. 1). The content of total *myo*-inositol in the material was approximately 2.433 $g \cdot kg^{-1}$, which is similar to the value determined in common wheat (2.454 $g \cdot kg^{-1}$) in our earlier study [18].

Dialysable *myo*-inositol is a pool of *myo*-inositol that passed the through pores in the dialysis membrane and was found in the buffer solution during simulated in vitro digestion. The lowest level of this compound was determined in raw grains of spelt and green spelt (83.43 $mg \cdot kg^{-1}$ and 57.43 $mg \cdot kg^{-1}$, respectively). Boiling resulted in the increase in this fraction, by 22 % on average. This was probably the effect of partial thermal hydrolysis of *myo*-inositol deposited in the form of phytates [31, 32].

The highest content of dialysable *myo*-inositol was found in spelt grains subjected to solid-state fermentation with *R. oligosporus*. The green spelt tempe was contained 141.95 mg of dialysable *myo*-inositol per kilogram (47% increase, as compared to boiled grains), whereas the value determined for tempe from mature grains was lower, 100.03 $mg \cdot kg^{-1}$ (37% increase, as compared to boiled grains). The comparison of these values to those of total *myo*-inositol in raw grains indicated the increase from 2.4–3.0 % to 4.0–6.0 %, which amounted to, on average, 75% increase in bioavailability of the said compound (Tab. 1).

The determined significant increase in bioavailable *myo*-inositol can be explained by the simultaneous hydrolysis of phytates and the release of *myo*-inositol as the final product through the action of phytases and phosphatases present

Tab. 1. Content and in vitro bioavailability of *myo*-inositol from spelt wheat (*Triticum spelta* L.) grains fermented with *Rhizopus oligosporus*.

Sample	Processing	Dialysable *myo*-inositol [$mg \cdot kg^{-1}$]	Total *myo*-inositol [$mg \cdot kg^{-1}$]	In vitro bioavailability [%]
Spelt wheat mature	Raw	57.43 ± 0.73 ^{a}	2428.08 ± 96.57	2.4
	Boiled	72.85 ± 3.28 ^{b}	2384.15 ± 172.93	3.1
	Tempe	100.03 ± 1.66 ^{e}	2454.65 ± 134.15	4.1
Spelt wheat immature	Raw	83.43 ± 4.07 ^{c}	2523.24 ± 38.02	3.3
	Boiled	96.44 ± 0.83 ^{d}	2501.40 ± 70.12	3.9
	Tempe	141.95 ± 3.45 ^{f}	2310.83 ± 69.24	6.1

Values in columns represent means ± standard deviation (n = 3). Values with different letters in superscript within columns are significantly different ($p < 0.05$).

in spelt grains and also produced by the fungal strain during fermentation [33, 34]. The thermal treatments applied (boiling prior to fermentation, followed by a 10 min steaming of the final product) were short and, as indicated in numerous studies, the activity of endogenous plant phytases and microbial enzymes in the material could have remained at a relatively significant level (residual activity of 20–80 %) [34, 35]. This could allow for further phytate degradation in the material during in vitro digestion.

CONCLUSIONS

One option for enriching processed cereal products with B-group vitamins is to introduce their purified forms into the raw material or a semi-finished product. However, a more advantageous solution, when bioavailability is concerned, is enriching the products with ingredients of natural origin. The results obtained in our study proved that solid-state fermentation of spelt grains with *R. oligosporus* has a beneficial effect on the content of selected bioactive components in the product. There was a 2.5-fold increase in the content of riboflavin and a 33% increase in the content of thiamine in the tempe, as compared to boiled grains. The in vitro bioavailability of riboflavin from the fermented products was high (83 % on average). Another positive result of fermentation was an increase in the bioactive *myo*-inositol in vitro availability, by 42 % on average, as compared to boiled spelt grains. In conclusion, the beneficial effect of the tempe-type processing of spelt wheat (*Triticum spelta* L.) on the content of selected B vitamins and *myo*-inositol confirmed the high potential of this organic cereal variety as a raw material for the production of functional foods.

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