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Prolonged tempe-type fermentation in order to improve bioactive potential and nutritional parameters of quinoa seeds

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ABSTRACT

The effect of 40 h solid-state fermentation with *Rhizopus oligosporus* on selected parameters of white and coloured quinoa was studied, as compared to standard (30 h) product and cooked seeds.

The reducing power (RP) and the activity against synthetic free radicals of standard tempe were higher by on average 140% (white) and 64% (coloured quinoa) than that of cooked seeds. The $^{\bullet}OH$ scavenging activity was increased by more than 7 fold (white), and over 2 fold (coloured quinoa). Prolongation of the fermentation caused further improvement in this potential, on average by 27% ($^{\bullet}OH$, RP) and 24% (DPPH$^{\bullet}$, $ABTS^{\bullet+}$ assays). The soluble phenols i.e. vanillic acid, protocatechuic acid and rutin levels in 40 h tempe were significantly higher than in cooked quinoa. Fermented products contained 470% (white) and on average 150% (coloured quinoa) more riboflavin and 100% more thiamine (white quinoa) than cooked seeds. The levels of total protein, free amino acids and proteins releasable during the *in vitro* digestion, were improved as a result of 40 h fermentation. The essential amino acids profile of quinoa tempe was consistent with the reference pattern.

The prolonged tempe-type fermentation of quinoa can be recommended as a method of the value-added food production.

1. Introduction

Quinoa (*Chenopodium quinoa* Willd.) is a pseudocereal originating from South America. This important ancient crop, known prior to 3000 B.C., was in later times classified as food for the poor, cultivated at a small scale for the domestic consumption only. The recent revival of its worldwide popularity was concluded in announcing 2013 as the International Year of Quinoa. Quinoa is currently grown in Bolivia, Peru (88% production and main exporters), Argentina, Chile, Colombia and Equador. However, attempts are being made to introduce this crop into Europe, North America, Asia and Africa. Quinoa seeds are a source of gluten-free protein with well-balanced amino acids profile, dietary fibre, polyunsaturated fatty acids as well as vitamins (niacin, riboflavin, α-tocopherol) and minerals (Ca, Fe, Mg). They also contain higher level of phenolic compounds than cereal grains, the majority of which are phenolic acids – vanillic and ferulic acids and their derivatives, as well as quercetin and kaempferol and their glycosides as main flavonoids (Hirose et al., 2010; Tang et al., 2015; Vega-Gálvez et al., 2010). In order to prepare quinoa for the consumption, the seeds must be subjected to hydrothermal treatment. During this process the partial loss of phenolic compounds occurs, due to their leaching into cooking water and/or partial breakdown, resulting in the lower antioxidant activity of a product (Dini et al., 2010).

Tempe-type fermentation of plant seeds with *Rhizopus oligosporus* may result in products of added functional value, as compared to conventional cooking. Moreover, fermented seeds are usually superior in terms of both nutritional and sensory parameters. The preparation of tempe-type products for the consumption is quick and easy, which allows to classify them as a convenient food (Owen and Owen, 2010). The traditional substrate for this fermentation are soaked, pre-cooked and dehulled legume seeds, especially soybeans, but legume-grain mixtures or cereal grains alone can also be used. This procedure requires shorter cooking

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time than the conventional process, because fungal activity results in partial digestion of seed tissue as well as decomposition of antinutrients. *Rhizopus* strains suitable for high-carbohydrate substrate fermentation should exhibit scarce amylolytic activity, in order to prevent the release of monosaccharides further metabolized by the fungus to organic acids (fumaric, citric, lactic), which would result in the undesirable sour taste of the product (Berg et al., 2007; Wang and Hesseltine, 1966).

During the fermentation of soy, the process must be stopped at a certain time when the substrate is uniformly overgrown with mycelium (usually 24–30 h) because its prolongation causes the ammonification of a product (Hedger, 1982). However, we found that in case of quinoa tempe obtained with *R. oligosporus* ATCC 64063 the extension of fermentation up to 40 h did not cause the disadvantageous sensory changes.

The purpose of the study was to apply the prolonged (40 h) tempe-type fermentation in order to enhance the bioactive potential of quinoa seeds, as compared to the standard (30 h) fermentation procedure and the conventional treatment (cooking). White, red and black seeds were used in order to compare the efficiency of the process for different quinoa varieties. Next, the modified tempe product was characterised according to the nutritional components as well as the *in vitro* protein and carbohydrates bioavailability.

2. Materials and methods

Quinoa varieties of white, red and black seeds (Bolivia origin), packaged by Bio Planet s.a., were purchased from a local health-food store in Krakow, Poland.

Rhizopus oligosporus ATCC 64063 strain recommended for cereal tempe-type fermentation (Berg et al., 2007) was grown for 12 days on potato dextrose agar (PDA) at 24 °C. Then, the spores suspension in sterile saline solution (8 g/L, supplemented with peptone (0.01 g/L) and Tween 80 (0.1 mL/L)) was obtained, and filtered three times through nylon net filters (mesh diameter 11 μm, Millipore, Tullagreen Carrigtwohill, Ireland) in order to remove the mycelium fragments. The spore density was obtained by spore counting method in Thoma chamber.

2.1. Cooking procedure

Quinoa seeds were rinsed with water and then boiled in tap water (1:3 m/v) for 15 min (white seeds) or 20 min (coloured seeds), considered as the traditional cooking time.

2.2. Fermentation procedure

Quinoa seeds (200 g) were rinsed with water, and pre-cooked in tap water (1:3 m/v, acidified to pH 4.5–5 with lactic acid to assure the proper conditions for mycelium growth) for 10 (white seeds) or 15 (coloured seeds) min. Next, they were dried on the surface with sterile cloth, cooled (<35 °C) and mixed thoroughly with *R. oligosporus* inoculum (10^4 spores per g). Inoculated material, of 68% moisture content, was tightly packed in sterile Petri dishes (diameter 10 cm) and incubated for 2 h at 35 °C to induce spores germination. Then the temperature was changed to 31 °C and the process was continued up to 30 (standard tempe - seeds uniformly overgrown with mycelium) and 40 (prolonged fermentation) hours. Fungal growth was stopped by steaming the fresh tempe (10 min).

All the obtained cooked and fermented samples were lyophilised and stored at 3 °C for further analysis. For the phenols content and antioxidant activity measurements, the samples were defatted according to the procedure described below.

2.3. Analytical methods

Antioxidant parameters and phenolic compounds were determined in acetone: water (1:1, v/v) and/or phosphate buffer (0.1 mol/L, pH 7.4) extracts. Buffer extracts were prepared by trituration of 0.5 g material with 15 mL of buffer (5 min) in a mortar, centrifugation (15,000 rpm, 20 min) and filtration of the supernatant. Acetone: water extracts were obtained by shaking of 1 g sample with 20 mL of acetone: water mixture for 3 h and further 18 h incubation (in the darkness), followed by centrifugation (15,000 rpm, 15 min) and filtration. 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH$^{\bullet}$) scavenging activity (Pekkarinen et al., 1999) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (ABTS$^{\bullet+}$) scavenging activity (Starzyńska-Janiszewska et al., 2014) were expressed as μg trolox/mg d.m. equivalents. Calibration curves were prepared in the range of 0–1.5 mM trolox. Hydroxyl radical neutralization was measured according to Marambe et al. (2008), and expressed as μg trolox/mg d.m. equivalents, with calibration curve prepared in the range of 0–10 mM trolox. Reducing power (Ardestani and Yazdanparast, 2007) was expressed as μg BHT/mg d.m. equivalents, with calibration curve prepared for 0–0.68 mM BTH. Soluble phenols (mg gallic acid/g d.m.) were measured by the reaction with Folin-Ciocalteu reagent as described in Starzyńska-Janiszewska et al. (2014). The separation of individual phenolic compounds present in acetone: water extracts was performed on reversed-phase column for high performance liquid chromatography (Luna C18, 250 mm × 4 mm i.d., Phenomenex, Torrance, CA, USA). Mobile phase was a mixture of water/glacial acetic acid (98:2, v/v) (solvent A) and methanol/glacial acetic acid (98:2, v/v). The elution gradient employed for polyphenols was: 85:15 (A/B) to 60:40 (A/B) in 30 min, 60:40 (A/B) to 25:75 (A/B) in 10 min and 25:75 (A/B) to15:85 (A/B) in the next 5 min. The flow rate was set at 1.2 mL/min. Eluates were monitored at 254 nm, 280 and 325 nm (Anesini et al., 2012). Rutin, vanillic acid and protocatechuic acid standards were from Sigma-Aldrich.

Dry matter was determined with a moisture analyzer (WPS 110S, Radwag, Radom, Poland). Total protein content (g/100 g d.m.) was evaluated in previously mineralized samples on the basis of nitrogen level (assay with Nessler reagent) and multiplied by 6.25 (Marczenko and Balcerzak, 1998). Crude fat content (g/100 g d.m.) was measured by gravimetric method after Soxhlet extraction (Soxtec™, 2055 fat extraction system, Foos analytical, Denmark) with diethyl ether (PN-EN ISO 11085:2010). Ash content was determined by using a muffle furnace (type Czylok SM-2002) at 550 °C (ICC- STANDARD No. 104/1). Total carbohydrates content (g/100 g d.m.) was obtained by difference between 100 and the sum of total ash, crude fat and total protein. Protein and carbohydrates *in vitro* bioavailability after pepsin and pancreatic digestion, as well as soluble proteins and reducing carbohydrates level were estimated as described in Starzyńska-Janiszewska et al. (2012). The protein and carbohydrates susceptibility to enzymatic hydrolysis was expressed as % total protein or carbohydrates, respectively, released from material during the *in vitro* digestion.

Individual amino acids were determined after the hydrolysis (6 M HCl with 5 g/L phenol at 110 °C for 24 h under an argon atmosphere) by the ion-exchange chromatography with post-column derivatization with ninhydrin using an automatic amino acid analyzer, according to standard protocol of manufacturer (Ingos, Czech Republic, Prague). Sulfur-containing amino acids were analyzed as oxidation products (cysteic acid and methionine sulfone) obtained by performic acid oxidation followed by standard hydrolysis with HCl. Tryptophan was not determined, as it is destroyed during acid hydrolysis, while asparagine and glutamine turn into aspartic and glutamic acids, respectively, and were determined in those forms. The content of free amino acids (g/