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Antioxidant properties of extracts from fermented and cooked seeds of Polish cultivars of *Lathyrus sativus*

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Abstract

Antiradical and total antioxidant activities of extracts from raw, prepared for inoculation, fermented (tempeh) and cooked seeds of grass pea (*Lathyrus sativus* Krab and Derek cultivars) were measured. Tempeh fermentation with *Rhizopus oligosporus* resulted in higher scavenging activity towards DPPH$^{\cdot}$ and ABTS$^{\cdot+}$ radicals which correlated well with the content of total phenols. In Derek cultivar, fermentation caused a significant inhibition of linoleic acid oxidation by methanol extracts. In buffer extracts the highest TAA values were observed in raw seeds. Cooking of seeds lowered RSA values as compared to fermentation, especially for the DPPH$^{\cdot}$ assay. Methanol and buffer extracts from cooked seeds showed prooxidant activity towards linoleic acid.

Keywords: Antiradical activity; *Lathyrus sativus*; *Rhizopus oligosporus*; Tempeh; Total antioxidant activity

1. Introduction

Grass pea is a legume plant widely consumed in developing countries of Asia and Africa. In Poland, its forms have only locally been grown in the Podlasie region. Great taste and nutritional value of the seeds together with little cultivation requirements have gained the interest of Polish scientists (Milczak & Masłowski, 1993) who in 1998 selected the first Polish grass pea cultivars – Derek and Krab with small and large seeds, respectively.

The factor that still reduces the interest of consumers is a toxic amino acid, β-Odap, present in grass pea seeds. Our preliminary research showed that the effective process of partial seed detoxification is tempeh fermentation with *Rhizopus* strains (data unpublished). Solid-state fermentation of legumes seeds is an ancient method that nowadays is still used for enhancing the nutritional and organoleptic qualities of legumes. Tempeh fermentation is a well known alternative to traditional methods of food processing like cooking, which cause partial loss of nutritionally valuable ingredients, e.g. vitamins.

An interesting application of solid-state fermentation is the production of foods enriched in non-enzymatic antioxidants. It has been proven that tempeh fermentation of legume seeds, especially soybeans, may increase the concentration of antioxidant phenols capable of scavenging free radicals and chelating metal ions (Sheih, Wu, Lai, & Lin, 2000). There are epidemiological studies showing a relationship between the consumption of products rich in phenols and a low incidence of diseases like certain forms of cancer, coronary heart disease or atherosclerosis (Randhir, Wattem, & Shetty, 2004). Apart from compounds with very strong antiradical properties, other ingredients of antioxidant activity for example aromatic amino acids and peptides (e.g. glutathione), are also present in fermented seeds. For consumers, only non-enzymatic antioxidants present in fermented foods are significant, as it has been shown that antioxidant enzymes do not add to the antioxidant value of tempeh (Fernandez-Orozco, Zieliński, & Piskuła, 2003). The most important is the overall antioxidant potential of the fermented product which includes combined and possible synergic activity of all compounds.

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The purpose of the study was to investigate if the fermentation with *Rhizopus oligosporus* DSM 1964 may enhance the antioxidant properties of Krab and Derek seeds. The objective of this research was also to compare the antioxidant value of tempeh with cooked seeds of grass pea.

To our knowledge, this study is the first that concerns tempeh made from Polish cultivars of *Lathyrus sativus* Derek and Krab and, thus, the antiradical and antioxidant activities of extracts obtained from this product are the main issue of investigations presented here.

2. Materials and methods

2.1. Materials

The seeds of *L. sativus* (Polish cultivars Derek and Krab) were obtained from the company 'Spójnia Hodowla i Nasiennictwo Ogrodnicze' in Nochowo, Poland. The starter for the tempeh fermentation (type B), containing *R. oligosporus* DSM 1964 culture, was purchased from 'Top Cultures', Zoersel, Belgium.

2.1.1. Tempeh production

L. sativus seeds (250 g) were cleaned with tap water, cooked for 30 min and then soaked for 18 h. Next, they were dehulled by hand, parted into halves and cooked for 30 min (with addition of 13 cm^3 of 6% vinegar). After drying, the seeds were cooled to 35 °C, supplied with 3.5 cm^3 of vinegar and mixed thoroughly with 0.975 g of *Rhizopus* starter culture. The inoculated material was put in perforated plastic bags, 3 cm in height, and fermented at 32 °C for 31 h (until a tight 'cake' was formed).

2.1.2. Cooking

Whole seeds were cleaned, soaked for 18 h and then cooked in tap water until soft (50 min for Krab and 40 min for Derek).

2.1.3. Sample preparation

The raw seeds (S) of both cultivars were ground in a seed mill (1 mm in mesh diameter). The cooked seeds (C), the seeds prepared for inoculation with *Rhizopus* starter (P) and the fermented product (tempeh, T) of both cultivars were dried at 60 °C for 24 h and ground in a seed mill. The flours from S, C, P and T were stored at 2–4 °C in closed vessels until analyzed.

2.2. Analytical methods

2.2.1. Reagents

1,1-Diphenyl-2-picryl-hydrazyl (DPPH); 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 97%; 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{\cdot+}$) diammonium salt, 98%; linoleic acid, 95%; polyoxyethylenesorbitan monolaurate (Tween 20) and Folin–Ciocalteu reagent were obtained from Sigma. Tannic acid and other reagents were of analytical grade from Chempur or Przedsiębiorstwo Odczynniki Chemiczne (POCh), Poland.

2.2.2. Radical scavenging activity (RSA)

The reduction of synthetic stable free radicals $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ by compounds present in flour extracts was monitored by a spectroscopic assay of discoloration of initial radicals' solutions. The radical scavenging activity of extracts was compared with the activity of Trolox solutions and expressed as μmol Trolox g^{-1} dm.

Scavenging of $DPPH^{\cdot}$ radical: 1.25 g of flour was shaken with 45 cm^3 of extraction mixture (96% ethanol, glycerin and distilled water 1:1:1 v/v) for 2 h at 60 °C. After centrifugation (15 min, 4000 rpm), the supernatant was made up to final volume of 50 cm^3 with distilled water. The $DPPH^{\cdot}$ radical scavenging activity was measured according to Pekkarinen, Stockmann, Schwarz, Heinonen, and Hopia (1999). A 0.05 cm^3 portion of the extract was mixed with 2.950 cm^3 of $DPPH^{\cdot}$ radical (0.1 mmol dm^{-3} in 80% methanol) and the absorption was measured at 516 nm after 5 min against an 80% methanol blank.

Scavenging of $ABTS^{\cdot+}$ radical: 0.125 g of flour was homogenized (supersonic homogenizer Labsonic P) with 5 cm^3 of phosphate buffer (0.1 mol dm^{-3}, pH 7.4) and centrifuged. The $ABTS^{\cdot+}$ radical scavenging ability was determined as described by Cano, Acosta, and Arnao (2003) with some modifications. To prepare the $ABTS^{\cdot+}$ solution, 10 mg of $ABTS^{\cdot+}$ was dissolved in 1.3 cm^3 of 0.0049 mol $K_2S_2O_8$ and 1.3 cm^3 of distilled water. After mixing, the tube was closed and left for 16 h at room temperature and then, stored at 2–4 °C. Just before analysis, $ABTS^{\cdot+}$ solution was diluted with phosphate buffer (0.1 mol dm^{-3}, pH 7.4) so that the absorbance of 0.7 ± 0.02 at 734 nm was achieved. The antiradical activity assay of extracts was performed in tubes tightly covered with aluminum foil. $ABTS^{\cdot+}$ (2 cm^3) solution and 200 μl of extract was mixed and incubated for 6 min at room temperature. The absorption of mixture was measured at 734 nm, with phosphate buffer as a reference.

2.2.3. Total antioxidant activity (TAA)

Total antioxidant activity was estimated according to Toivonen and Sweeney (1998). The method is based on the spectrophotometric assay of primary products of linoleic acid peroxidation (conjugated dienes) which may be inhibited or stimulated by compounds present in extracts.

The assay was conducted in extracts of two kinds. The methanol extracts were obtained by homogenizing of 1.25 g of flour with 7 cm^3 of 80% methanol for 5 min. After centrifugation the extracts were filled up to final volume of 4.5 cm^3 with 80% methanol. The buffer extracts were obtained in the same manner, except that 8 cm^3 of phosphate buffer pH 7.0 (0.02 mol dm^{-3}) was used for homogenization and the centrifuged extracts were made up to total volume of 5.5 cm^3 with buffer solution. The solution of linoleic acid was prepared daily by diluting 0.56 g of