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The effect of germination process on the superoxide dismutase-like activity and thiamine, riboflavin and mineral contents of rapeseeds

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Abstract

Seeds of double low oilseed rape variety Mango (*Brassica napus*, var. oleifera) were subjected to a 7-day germination at 25 °C and 95% moisture content in darkness in a conditioning cabinet. The effects of the germination process on the superoxide dismutase-like activity (SOD-like activity), thiamine (vitamin B_1) and riboflavin (vitamin B_2) and minerals, such as Ca, Mg, Cu, Fe and Mn, were studied. Correlations between individual mineral contents, vitamin B_1 and B_2 contents, and the ability of phosphate buffered saline (PBS) extracts from germinated rapeseed to scavenge superoxide anion radicals *in vitro* were also investigated. SOD-like activity showed a gradual increase after the second day of germination, reaching a maximum level on the sixth day, and remained almost constant up to the end of the germination period. During germination, thiamine underwent a progressive decrease up to the sixth day, reaching a constant level between the sixth and the seventh day. In contrast, riboflavin content increased throughout the germination period up to the fifth day, and after that a constant level was observed. Levels of Ca and Mg were almost constant up to the fourth day and after that an increase of these minerals was observed. Cu and Mn increased during the germination process, and retentions of 33% and 22%, respectively, were observed at the end of germination. Fe content dropped after 1 day of germination and from there onward it started to increase gradually and an 18% retention was observed in 7-day germinated seeds. Positive correlations between SOD-like activity and riboflavin ($r = 0.87$), Cu ($r = 0.74$) and Mn ($r = 0.87$) were found during rapeseed germination.

Keywords: Rapeseed; Germination; Thiamin; Riboflavin; Minerals; Superoxide dismutase-like activity

1. Introduction

A plant-based diet – focusing mainly on vegetables, fruits and whole grains – has become one of the most important guidelines for lowering the risk of human diseases (Lawrence & Machlin, 1995). People should consume several hundred grammes of plant-based diet a day since it is a good source of nutrients and dietary fibre.

Cereal grains and legume seeds are usually submitted to technological processes, such as fermentation and germination, in order to improve the nutritive value of the final products (Yang, Basu, & Ooraikul, 2001; Sadowska, Fornal, Vidal-Valverde, & Frias, 1999; Trugo, Donangelo, Trugo, & Knudsen, 2000; Bartolome, Estrella, & Hernandez, 1997). Germination is an economical and simple method for improving the nutritive value, and several studies have reported higher levels of nutrients and lower levels of antinutrients in sprouts compared to the ungerminated seeds (King & Perwastien, 1987; Raman, 1984; Honke, Kozłowska, Vidal-Valverde, Frias, & Górecki, 1998).

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Germination starts with the uptake of water (imbibition) by the quiescent dry seed and terminates with the emergence of the embryonic axis, usually the radicle. It is a time of intense metabolic activity, involving subcellular structural changes, respiration, macromolecular syntheses and, finally, cell elongation. Establishment of the seedling occurs following germination and its growth is initially supported by metabolites produced by the hydrolysis and conversion of the major stored reserves proteins, carbohydrates and lipids.

Rapeseeds occupy a prominent position among oilseed crops on a worldwide basis. Like other oilseeds, they are mainly utilized as animal feed for the protein and lipid components. However, some trials aim to include the rapeseed sprouts in human nutrition because of their content of glucosinolates (Kozłowska, Troszyńska, Zieliński, Buciński, & Lamparski, 2002), ascorbic acid (Zieliński, Buciński, & Kozłowska, 2002), tocopherols (Zieliński & Kozłowska, 2003), reduced glutathione (Zieliński, Mudway, Kozłowska, & Kelly, 2002), dietary fibre (Zieliński, Frias, Piskuła, Kozłowska, & Vidal-Valverde, 2005), inositol hexaphosphates and higher total antioxidant status (Kozłowska, Zieliński, Buciński, & Piskuła, 2003) when compared to the ungerminated rapeseeds.

The aim of this work was to study the effects of germination process on the SOD-like activity during rapeseed germination in order to obtain ready-to-eat sprouts with high antioxidant activity. The content of two water-soluble vitamins (thiamine and riboflavin) and several minerals (Ca, Mg, Cu, Fe and Mn) during germination of rapeseeds were also determined. The ready-to-eat rapeseed sprouts can be offered to consumers as a functional food with added value.

2. Materials and methods

2.1. Reagents

Thiamine and riboflavin standards were obtained from Merck (Darmstadt, Germany). Bovine serum albumin (fraction V; BSA) was purchased from Sigma (Sigma Chemical Co., St. Louis, MO, U.S.A.). The superoxide dismutase kit (RANSOD, Cat No SD 125) was from Randox Laboratories Ltd, (Crumlin, Co. Antrim, UK). Nitric acid and perchloric acid were from Merck (Darmstadt, Germany). The standards of elements being analysed were purchased from BDH (Poole, UK). All other chemicals were of reagent-grade quality and were from POCh, Gliwice, Poland.

2.2. Samples

Double low oilseed rapeseeds of Mango variety (*Brassica napus*, var. oleifera) were obtained from a local plant breeding station in the North-East Poland. The seeds were stored at room temperature in polyethylene bags until germination.

2.3. Seed germination

Twenty five grammes of rapeseeds were soaked in 125 ml of cooled, boiled water and shaken every 30 min. After 4 more hours of soaking, the water was drained off and the seeds were transferred to an incubator (Cliambic Cabinet, model Economic Deluxe EC00-065, Snijders Scientific b.v, Netherlands). The seeds were germinated in the dark at 25 °C and 95% moisture content for 7 days. The sprouts were layered over a moist filter paper (qualitative medium-speed filter paper). The germinated seeds were removed from the incubator at 24-hour intervals, frozen in liquid nitrogen, lyophilized and stored in a freezer until used. The germination was carried out in triplicate.

2.4. Preparation of extracts for determination of SOD-like activity

The lyophilized seeds and sprouts were ground and then subjected to extraction, in triplicate, with phosphate buffered saline (PBS, pH 7.4, 5 ml per 0.5 g of sample) for 2h by shaking at room temperature. They were then centrifuged at $5{,}000 \times g$ (centrifuge type MPW – 360, Factory of Precise Mechanics, Warsaw, Poland) and the fresh supernatants were used to determine their ability to scavenge superoxide anion radicals and the content of soluble proteins.

2.5. Analysis methods

2.5.1. Determination of thiamine and riboflavin

Vitamins B_1 and B_2 were extracted and subsequently analysed by HPLC according to Frias, Prodanov, Sierra, and Vidal-Valverde (1995).

2.5.2. Determination of minerals

The contents of Ca, Mg, Cu, Fe and Mn were determined after digestion of the samples with a mixture of nitric and perchloric acids (3:1, v/v) (Digeston System 20, Tecator, USA). The quantitative analyses were performed by flame-atomic absorption spectrometry (Pye Unicam SP 939 spectrometer, UK).

2.5.3. Determination of SOD-like activity

The superoxide scavenging activity of the phosphate-buffered saline sprouts extracts, collected at 24-hour intervals during germination, was measured according to the method provided with the superoxide dismutase kit (RANSOD). The SOD-like activities of the respective extracts were calculated as SOD units/ml and finally the data were converted to units/milligramme of soluble protein. The assays were performed in a spectrophotometer set at 37 °C (UV-160 1PC with CPS-Controller, Shimadzu, Japan). The test required 50 μl of sample, with a read time of 3 minutes. The concentration of soluble proteins in the phosphate-buffered saline extracts was measured using the Bradford protein microassay with bovine serum albumin (BSA) as the standard (Bradford, 1976).