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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).

2.6. Statistical analysis

Data were subjected to multifactor analysis of variance (ANOVA) using the least-square difference test with the Statgraphic 5.0 Program (Statistical Graphic, Rockville, Md., USA) and multiple correlation using Statistica 5.1 Program (Statsoft, Tulsa, Okla, USA) for Windows using a PC-Pentium.

3. Results and discussion

In this paper, a new aspect was studied of the effect of germination on the superoxide-scavenging-like activity of sprouts. Also, the concentrations of thiamine and riboflavin in germinated rapeseed sprouts were determined by HPLC, which allowed rapid separation and quantification of thiamine and riboflavin with high sensitivity and reliability. Atomic absorption spectrometry was used to quantify Ca, Mg, Cu, Fe and Mn in ungerminated and germinated rapeseeds. The correlation between superoxide-scavenging-like activity of sprouts and vitamin B_1, B_2 and some mineral content was determined.

Dry matter decreased gradually during the seven day period of germination but the highest loss was observed up to the fourth day (Fig. 1). According to Troszyńska, Lamparski, and Kozłowska (2002), ready-to-eat rapeseed sprouts were obtained after 4 days under dark conditions, and those seeds contained only 15% of dry matter. The loss of dry matter during sprouting is mainly due to the imbibition of the seeds during germination and degradation and oxidation of seed components during respiration to provide energy for the increased metabolic functions.

An almost linear reduction in thiamine content during the rapeseed germination up to the sixth day was observed, reaching a constant level of thiamine until the end of the process (Fig. 2). The concentration of thiamine in ready-to-eat sprouts (after four days of germination) was 57% of that of the raw seeds when data were expressed on dry matter basis. On the other hand, a 100 g portion of fresh ready-to-eat rapeseed sprouts contained about 43 μg of thiamine and represented only 8.7% of the amount found in the raw seeds. This finding indicates that a 100 g portion

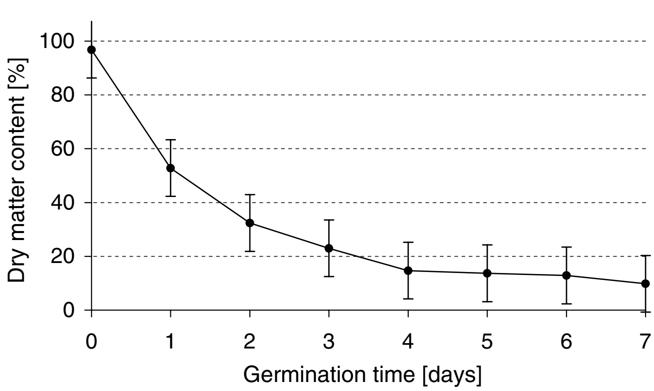

Fig. 1. The changes in dry matter content during rapeseed germination.

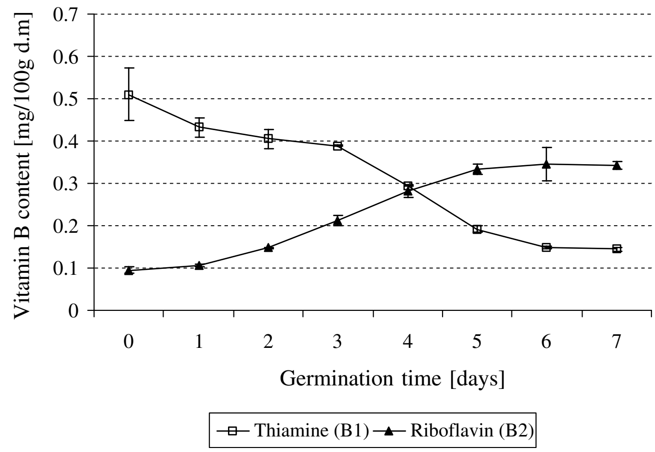

Fig. 2. The changes in thiamine and riboflavin contents during rapeseed germination.

of fresh sprouts may supply less than 10% of the required dietary vitamin B_1 for humans. The thiamine requirement of an individual is influenced by age, energy and carbohydrate intake and body weight. The Food and Nutrition Board, on the basis of considerable evidence, recommends 0.5 mg/1000 kcal (4184 kJ) (National Research Council, 1989). Because there are some data implying that older people use thiamine less efficiently, it is recommended that they maintain an intake of 1 mg/day even if they consume less than 2000 kcal (8368 kJ daily). Since thiamine requirement increases during pregnancy and lactation, an additional 0.4 mg/day is recommended during pregnancy and 0.5 mg/day during lactation. Thiamine content of ready-to-eat sprouts was about three-fold lower when compared to the four day-germinated legume and cereal grains (Bustorf-Hirsch, 1997).

Riboflavin content of raw rapeseeds was 0.095 mg/100 g d.m. In contrast, riboflavin content in sprouts increased gradually throughout the germination period up to the fifth day; after that a constant level was observed until the end of the process (Fig. 2). Moreover, the ready-to-eat sprouts provided an approximately three-fold higher riboflavin content than did the raw seeds, when data were expressed on a dry matter basis. Riboflavin content of ready-to-eat sprouts was about three-fold lower than that of the legume seeds and 4 day germinated cereal grains (Bustorf-Hirsch, 1997). Riboflavin daily intake is essential and the recommended daily allowances (RDAs) are 0.4–0.5 mg/day for infants, 0.8–1.2 mg/day for children, 1.5–1.7 mg/day for males and 1.3 mg/day for females (National Research Council, 1989). An additional 1.4 and 1.2 mg/day is recommended for people over 50 years old, 1.8 mg/day during lactation and 1.6 mg/day during pregnancy. Since a 100 g portion of fresh ready-to-eat germinated seeds contained about 42 μg of riboflavin, the intake of a fresh portion can supply about 10% of the currently established RDA for humans.

Despite legume sprouts being more popular, germinated rapeseed has not been fully characterized for its mineral

Table 1
The changes of mineral content in germinated rapeseed

Day of germination	Ca [mg/g d.m.]	Mg [mg/g d.m.]	Cu [μg/g d.m.]	Mn [μg/g d.m.]	Fe [μg/g d.m.]
0	4.46 ± 0.02^{abc}	2.89 ± 0.02	3.69 ± 0.03	36.1 ± 0.61	79.1 ± 0.31^{ab}
1	4.31 ± 0.22^{a}	3.08 ± 0.07	4.41 ± 0.15	37.5 ± 0.26	71.5 ± 0.80
2	4.50 ± 0.07^{b}	3.15 ± 0.04	4.47 ± 0.14	37.8 ± 0.85	73.4 ± 1.86
3	4.66 ± 0.19^{c}	3.30 ± 0.10	4.52 ± 0.12	38.9 ± 1.27	75.1 ± 0.71
4	4.79 ± 0.12	3.34 ± 0.05	4.61 ± 0.29	39.4 ± 0.90	75.8 ± 2.41
5	4.96 ± 0.11	3.51 ± 0.11	4.60 ± 0.40	40.6 ± 0.90	77.0 ± 2.25^{a}
6	5.18 ± 0.15	3.52 ± 0.07	4.67 ± 0.29	40.8 ± 0.79	78.4 ± 0.76^{b}
7	5.82 ± 0.19	3.83 ± 0.13	4.93 ± 0.27	44.4 ± 2.81	93.4 ± 2.15

(*) Mean values ± standard deviation of 3 determinations. The same superscript in the column for each element means no significant difference between the time of germination on "0" day and the time of germination from 1^{st} up to 7^{th} day ($P \leqslant 0.05$).

content. Data for microelements in the germinated rapeseeds are compiled in Table 1. The levels of Ca and Mg were not modified during germination up to the fourth day and, after that time, the increase of minerals was gradual to the end of the process, and a 30% rise was observed after a 7-day germination compared to the raw seeds (on dry matter basis). Among the analysed microelements, an increase in Cu and Mn was found, reaching higher levels of 33% and 22% respectively, in 7-day germinated seeds compared to the ungerminated ones. This finding is in agreement with previous data reported by Lintschinger et al. (1997) related to the course of cereal grain germination. Iron (Fe) content decreased sharply during the first day of germination and from there onward it increased gradually and, at the final germination period, a rise of 18% of Fe was observed. In general, the results indicate that sprouts are a richer source of these microelements than are breakfast cereals (Morris & Hill, 1995).

The superoxide dismutase (SOD)-like activity of rapeseed sprouts increased significantly when compared to the raw seeds (Fig. 3). SOD-like activity after 4 days of germination was 2-fold higher (when sprouts were ready-to-eat) and almost three-fold higher than on the last day of germination. This increase was, in part, associated with the large reduction of soluble protein content during the entire germination period. The soluble protein content, expressed

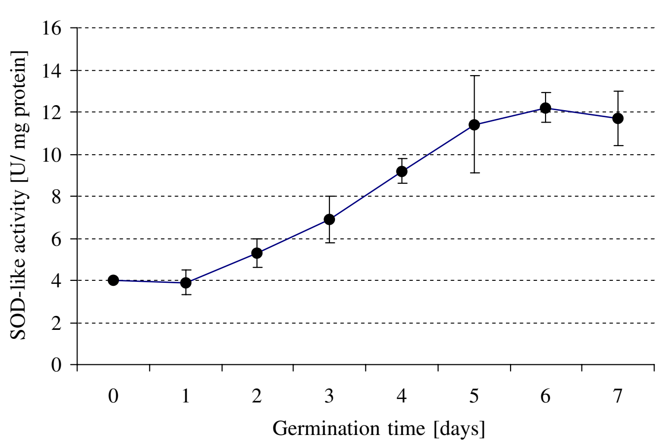

Fig. 3. The changes of SOD-like activity during rapeseed germination.

on dry matter basis, decreased in a linear fashion with only 46% present after 4 days of germination and 30% on the seventh day. The changes of SOD-like activity during the germination were possitively correlated with Cu ($r = 0.74$; $P \leqslant 0.05$) and Mn ($r = 0.87$; $P \leqslant 0.05$). Recently, it has been shown that apoplastic washing fluid isolated from foliage of *Avena sativa* L., *Hordeum vulgare* L., *Nicotiana tabacum* L., *Picea abies, Pinus sylvestris* L., and *Plantago major*, displays SOD-like activity (Streller & Wingsle, 1994; Vanacker, Carver, & Foyer, 1998; Vanacker, Foyer, & Carver, 1999). It has been proposed that apoplastic SOD may play a vital role in the lignification of the cell wall by supplying H_2O_2 to peroxidase and preventing peroxidase inactivation by superoxide anion radicals ($O_2^{\cdot -}$) (Ogawa, Kanematsu, & Asada, 1997). Particularly interesting was the relationship between increased riboflavin content and SOD-like activity ($r = 0.87$; $P \leqslant 0.05$). In contrast, a negative correlation was noted between SOD-like activity and thiamine content during germination ($r = -0.81$; $P \leqslant 0.05$).

4. Conclusions

Germinated rapeseeds have the ability to scavenge superoxide anion radicals and this property is clearly associated with riboflavin content of sprouts. Moreover, rapeseed sprouts are a good source of thiamine and riboflavin, despite having lower contents than have legumes or cereal sprouts. Moreover, rapeseed sprouts had well balanced mineral composition, and Cu and Mn might have played a role in the observed increase of superoxide dismutase-like activity during sprouting.

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References

Bartolome, B., Estrella, I., & Hernandez, T. (1997). Changes in phenolic compounds in lentils (Lens culinaris) during germination and fermentation. *Zeitschrift für Lebensmittel- Untersuchung und -Forschung A, 205*, 290–294.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*, 248–254.

Bustorf-Hirsch, M., (1997). Kiełki – źródło życia i zdrowia. Oficyna Wydawnicza SPAR, 9-9.

Frias, J., Prodanov, M., Sierra, I., & Vidal-Valverde, C. (1995). Effect of light on carbohydrates and hydrosoluble vitamins of lentils during soaking. *Journal of Food Protection, 58*, 692–695.

Honke, J., Kozłowska, H., Vidal-Valverde, C., Frias, J., & Górecki, R. (1998). Changes in quantities of inositol phosphates during maturation and germination of legume seeds. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung A, 206*, 279–283.

King, R. D., & Perwastien, P. (1987). Effects of germination on the proximate composition and nutritional quality of winged bean (*Psophocarpus tetragonolobus*) seeds. *Journal of Food Science, 52*, 106–108.

Kozłowska, H., Troszyńska, A., Zieliński, H., Buciński, A., & Lamparski, G. (2002). The use of rapeseedd for sprouts production in human nutrition. *Oilseed Crops, XXIII*(1), 165–173.

Kozłowska, H., Zieliński, H., Buciński, A., & Piskuła, M. K. (2003). Bioactive compounds in rapeseed sprouts. *Oilseed Crops, XXIV*, 31–39.

Lawrence, J., & Machlin, Ph. D. (1995). Critical assessment of the epidemiological data concerning the impact of antioxidant nutrients on cancer and cardiovascular disease. *Critical Reviews in Food Science and Nutrition, 35*, 41–50.

Lintschinger, J., Fuchs, N., Moser, H., Jager, R., Hlebeina, T., Markolin, G., & Gossler, W. (1997). Uptake of various trace elements during germination of wheat, buckwheat and quinoa. *Plant Foods for Human Nutrition, 50*, 223–237.

Morris, E. R., & Hill, A. D. (1995). Inositol phosphate, calcium, magnesium, and zinc contents of selected breakfast cereals. *Journal of Food Composition and Analysis, 8*, 3–11.

National Research Council., (1989). National Academy of Sciences, Washington, DC.

Ogawa, K., Kanematsu, S., & Asada, K. (1997). Generation of superoxide anion and localization of CuZn-superoxide dismutase in the vascular tissue of spinach hypocotyls: their association with lignification. *Plant Cell Physiology, 38*, 1118–1125.

Raman, A. H. Y. A. (1984). Improvement of nutritive value in corn for human nutrition. *Food Chemistry, 13*, 17–23.

Sadowska, J., Fornal, J., Vidal-Valverde, C., & Frias, J. (1999). Natural fermentation of lentils. Functional properties and potential in bread-making of fermented lentil flour. *Nahrung, 43*, 396–401.

Streller, S., & Wingsle, G. (1994). Pinus sylvestris L. needles contain extracellular CuZn superoxide dismutase. *Planta, 192*, 195–199.

Troszyńska, A., Lamparski, G., & Kozłowska, H. (2002). Sensory quality of sprouts of selected cruciferous species. *Polish Journal of Food and Nutrition Sciences, 11/52*(SI 1), 138–141.

Trugo, L. C., Donangelo, C. M., Trugo, N. M. F., & Knudsen, K. E. B. (2000). Effect of heat treatment on nutritional quality of germinated legume seeds. *Journal of Agricultural and Food Chemistry, 48*, 2082–2086.

Vanacker, H., Foyer, C. H., & Carver, T. L. (1999). Changes in apoplastic antioxidants induced by powdery mildew attack in oat genotypes with race non-specific resistance. *Planta, 208*, 444–449.

Vanacker, H., Carver, T. L., & Foyer, C. H. (1998). Pathogen-induced changes in the antioxidant status of the apoplast in barley leaves. *Plant Physiology, 117*, 1103–1109.

Yang, F., Basu, T. K., & Ooraikul, B. (2001). Studies on germination and antioxidant contents of wheat grain. *International Journal of Food Science and Nutrition, 52*, 319–330.

Zieliński, H., Buciński, A., & Kozłowska, H. (2002). Monitoring of the vitamin C content in germinating cruciferae seeds by HPLC. *Polish Journal of Food and Nutrition Sciences, 11/52*(SI 1), 142–146.

Zieliński, H., Frias, J., Piskuła, M. K., Kozłowska, H., & Vidal-Valverde, C. (2005). Vitamin B_1 and B_2, dietary fiber and minerals content of Cruciferae sprouts. *European Food Research and Technology, 221*, 78–83.

Zieliński, H., & Kozłowska, H. (2003). Content of tocopherols in cruciferae sprouts. *Polish Journal of Food and Nutrition Sciences, 12/53*(4), 25–31.

Zieliński, H., Mudway, I., Kozłowska, H., & Kelly, F. J. (2002). Impact of germination on glutatione content in cruciferous seeds. *Polish Journal of Food and Nutrition Sciences, 11/52*(SI 1), 68–72.