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Inositol phosphate content and trypsin inhibitor activity in ready-to-eat cruciferous sprouts

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Abstract

Four cruciferous seeds (small radish, radish, white mustard and rapeseed) were germinated in order to study the fate of inositol hexaphosphate (IP-6, phytic acid) and activity of trypsin inhibitor (TIA). Reduction in the content of phytic acid depended on the time of germination. After four days of germination, when sprouts were ready-to-eat, the amount of this compound was about 50% lower in three out of the four seeds evaluated. Next, a sharp reduction in phytic acid occurred during thermal treatments (pasteurization and sterilization) of germinated rapeseed and radish sprouts. In thermal processes, a decrease in inositol hexaphosphate content was accompanied by the appearance of lower forms of inositol phosphates: IP-5, IP-4 and IP-3.

The analysis of TIA, in rapeseed and radish seeds, in four-day sprouts and in these sprouts after thermal treatment, showed that only thermal processes caused complete disappearance this activity.

Keywords: Cruciferous seeds; Germination; Sprouts; Phytic acid; Inositol phosphates; Trypsin inhibitors

1. Introduction

It is well known that the consumption of a plant-based diet, mainly vegetables, fruits and whole grains, is recommended as one, of the ways to lower the risk of human diseases (Cadenas & Packer, 2002), and this approaches consumer demand for healthy and functional foods (Roberfroid, 2000). One valuable but still under-appreciated dietary supplement is sprouts which may be considered to be a functional food and to meet consumer demands as well. To date studies into the chemical composition of sprouts obtained from different seeds and cereals have indicated their high nutritive value and shown that they contain biologically-active, positive components which can improve health and well being (King & Perwastien, 1987; Price, 1988). It has been proved that sprouts have a higher nutritive value than seeds and that the process of their production is inexpensive and simple (Finley, 1978; Frías, Díaz-Pollán, Hedley, & Vidal-Valverde, 1996; Vidal-Valverde et al., 2002). Hence, Kuo & Van Middlesworth (1988) suggested popularisation of their consumption. Ever since, sprouts have become a component of everyday day in Japan (Kuo & Van Middlesworth, 1988). Additionally, taste and texture of sprouted seeds can be further modified compared with the ungerminated seeds (Finley, 1978).

Protein-rich seeds (of oil plants and legumes) contain substances that, until recently, have been acknowledged as anti-nutrients, including phytic acid (inositol hexaphosphate) and trypsin inhibitors (Frías, Díaz-Pollán, Hedley, & Vidal-Valverde, 1995; Honke, Kozłowska, Vidal-Valverde, Frias, & Górecki, 1998). As reported in

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ample studies, apart from some negative functions, e.g. affecting the bioavailability of minerals in the case of phytic acid (Lönnerdal, Sandberg, Sandström, & Kunz, 1989) and lowering the protein digestibility in the case of trypsin inhibitors (Liener & Kakade, 1978), these compounds have also been claimed to have some potential health benefits. Phytic acid – due to its chelating properties – binds some macro- and microelements responsible for generation of free radicals (Graf & Eaton, 1990), and also lowers blood glucose, reduces cholesterol and triacylglycerols, and reduces the risk of cancer and heart diseases (Graf & Eaton, 1993; Shamsuddin, 1995). Moreover, epidemiological studies have identified legumes as possible protective agents in the decreased occurrence of breast, colon and prostatic cancers in vegetarian populations. The association of legume seeds rich in proteinase inhibitors, including trypsin inhibitors, with prevention of human cancers, stimulated work on the possible action of proteinase inhibitors as cancer chemopreventive agents (Birk, 1994).

So far, the chemical composition of sprouts from grain legume seeds has been best recognised (Bannerjee, Rohatgi, & Lahin, 1954; Raman, 1984; Reddy, Pierson, Sathe, & Salunkhe, 1989; Prodanov, Sierra, & Vidal-Valverde, 1997). While searching for new sources of functional food especial attention has been paid to sprouts from rapeseed (Cruciferae family) that have been increasingly used in human diets. The sprouts may thus become a potential source of nutritious food or become a food ingredient, It has been reported that rapeseed sprouts contain glucosinolates (Kozłowska, Troszyńska, Zieliński, Buciński, & Lamparski, 2002), ascorbic acid (Zieliński, Buciński, & Kozłowska, 2002), tocopherols (Zieliński & Kozłowska, 2003), reduced glutathione (Zieliński, Mudway, Kozłowska, & Kelly, 2002), and have higher total antioxidant status (Zieliński, Piskuła, Buciński, & Kozłowska, 2003a) than raw seeds. Moreover, radish sprouts have been shown to have the highest hydroxyl radical scavenging potency among 11 kinds of commonly available vegetables (Takaya, Kondo, Furukawa, & Niwa, 2003).

Data compiled so far on bioactive compounds in sprouts obtained from Cruciferae seeds, do not show the behaviour of inositol hexaphosphate and trypsin inhibitors during germination of seeds under industrial production conditions and on the effect of thermal treatments on these compounds. Therefore, the aim of this study was to bridge this gap.

2. Material and methods

2.1. Samples

Cruciferous seeds were obtained from a local plant breeding station in the North-East of Poland. The samples included: rapeseed (*Brassica napus* var. *oleifera*), radish seeds (*Raphanus sativus* L.), small radish seeds (*R. sativus* var. *sativus*), and white mustard seeds (*Sinapis alba* L.).

2.2. Seed germination

Cruciferous seeds (25 g) were soaked in distilled water (125 ml) at room temperature and shaken every 30 min. After 4 h, the water was drained off and the seeds were transferred to an incubator (Cliambic Cabinet, Economic Deluxe ECOO-065 model, Snijders Scientific, Netherlands). The seeds were germinated in darkness and/or light at 25 °C for 7 days. The seeds were layered over a moist filter paper (qualitative medium-speed filter paper) to one-third of the depth of the paper. Seeds from each species were removed from the incubator every 24 h, frozen in liquid nitrogen and lyophilized for further analysis. The germination was carried out in triplicate.

2.3. Thermal treatment

Fresh 4-day-germinated (in dark conditions) rapeseeds (*B. napus* var. *oleifera*) and radish seeds (*R. sativus* L.) were canned as follows: 100 g of sprouts were put into 150 ml jars and covered with water containing 2 g of citric acid, 24 g of sugar and 6 g of salt per litre. Jars were submitted to preserving procedures, such as pasteurization (95 °C for 30 min) and sterilization in a vertical steam autoclave (ASV, SMS, Poland) at 1 and 1.5 atm, for 30 min. After treatments, the contents were frozen in liquid nitrogen and lyophilized for further analysis. The canning process was carried out in triplicate.

2.4. Determination of inositol phosphates (IP-6 to IP-3)

Inositol phosphates were analysed by HPLC according to the methods of Sandberg and Ahderinne (1986) and Sandberg et al. (1989) using a Shimadzu chromatograph (LC-10 AD pump, refractometric detector RID-6A, CTO 6A column oven) and a Nova-Pak C_{18} column (Waters). The mobile phase was a mixture of methanol and 0.05 M formic acid (51/49, v/v) and 1.5 ml/100 ml TBA-OH. The flow rate was 0.4 ml/min and injections were made with a 20-μl loop. Sodium phytate (Merck) was used as an external standard.

2.5. Trypsin inhibitor activity

The activity of trypsin was determined in rape and radish seeds, in ready-to-eat 4-day sprouts and these sprouts after thermal treatment. Their trypsin inhibitor activity (TIA) was determined according to the method of Kakade, Rackis, McGhee, and Puski (1974), as modified by Valdebouze, Bergeron, Gaborit, and Delort-Laval (1980). Trypsin inhibitor activity was expressed

as trypsin inhibitor units per milligramme of the sample in the dry matter (TlU/mg d.m.).

2.6. Statistical analysis

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

3. Results and discussion

The influence of the time of germination on the kinetics of inositol hexaphosphate (IP-6-phytic acid) content was determined under light and dark conditions for four cruciferous seeds: radish seeds – *R. sativus* L., small radish seeds – *R. sativus* var. *sativus*, rapeseed – *B. napus* var. *oleifera* and white mustard seeds – *S. alba* L.

Fig. 1 shows the kinetics of IP-6 in seeds germinated for seven days under both conditions and analysed every 24 h. Raw seeds contained from 38.3 to 47.9 μmol/g d.m. of IP-6, and this amount decreased by around 50% after 4 days of germination, with the exception of the small radish seeds where a 77% drop in IP-6 content was observed (only under dark conditions). Further progressive fall in phytic acid was observed when germination time increased up to seven days. Consequently, after seven days of the experiment, the content of IP-6 reached as little as ca. 10%, with the exception of white mustard seeds, where it was higher by ca. 5%. The lowest IP-6 degradation appeared in white mustard seeds, perhaps because these raw seeds showed the highest content of this compound. However, the degradation patterns were similar for all the studied Cruciferae species and a close correlation between germination time and the content of IP-6 was found ($r = -0.99$) for all studied sprouts. A successive decrease in IP-6 content upon seed germination results from the absorption of micro- and macroelements by a plant from the element reservoir in seeds, namely IP-6 (Bewley & Black, 1982). Only limited data have been found on the effect of germination on the IP-6 content in Cruciferous seeds (Zieliński, Piskuła, Buciński, & Kozłowska, 2003b). In other seeds, such as legumes, germination has been found to bring about a large reduction in IP-6 and an increment in the content of lower inositol phosphates (Honke et al., 1998), which has not been observed during germination of the seeds examined. Reddy et al. (1989) have reported that the decrease in IP-6 occurring during germination is due to the increase in the activity of phytase.

From a practical point of view, a lack of significant differences reported in IP-6 behaviour during germination, under either dark or light conditions, seems very

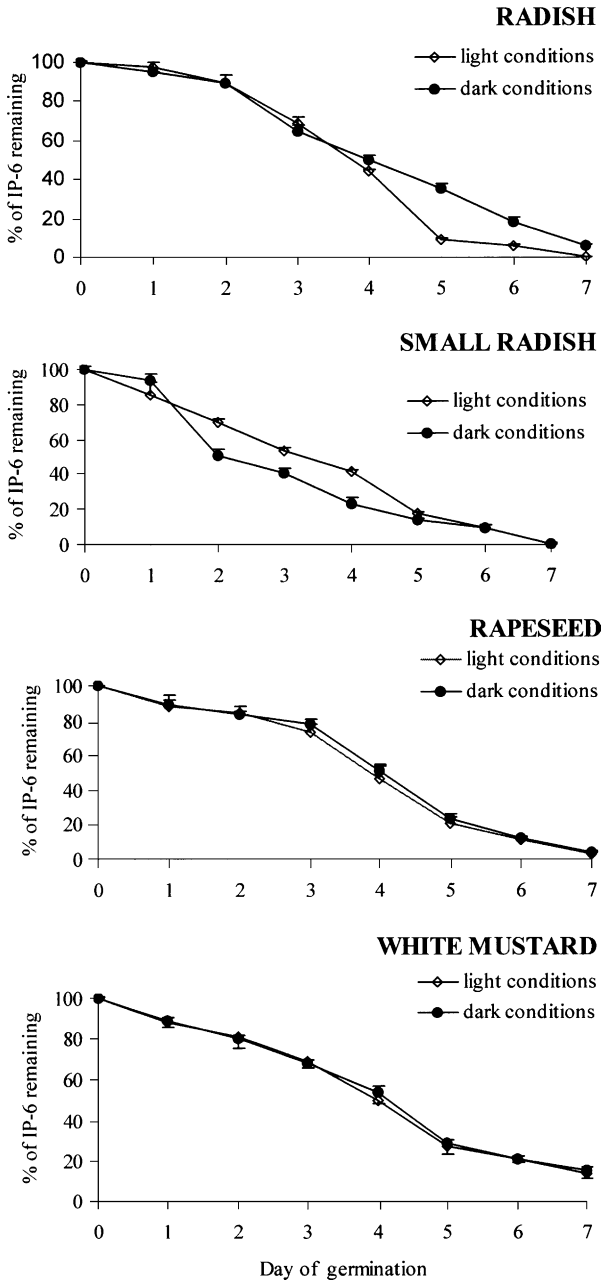

Fig. 1. Comparision of changes in inositol hexaphosphate (IP-6) content during germination of radish, small radish, rapeseed and white mustard seeds under light and dark conditions. Results are expressed as percent of change in content, with assumption that content at day 0 (before germination) was 100%. Each point represents mean ($n = 3$) of converted content of IP-6 at a specific time within germination protocol.

interesting. The only exception was small radish seeds (after 4 days of germination) in which a lower IP-6 content was observed when germination was carried out in darkness. Recently, Troszyńska, Lamparski, and Kozłowska (2002) have shown that germination of Cruciferous seeds for 4 days under dark conditions gives optimal sensory characteristics when compared with the same sprouts germinated with light. On the basis

of these results, a further study of the influence of pasteurization and sterilization processes on inositol phosphates and TIA was conducted on the two species selected: rapeseed and radish sprouts obtained after 4 days of germination in the dark.

Fig. 2 shows a decrease in IP-6 content after both investigated thermal treatments. The pasteurization process yielded a greater IP-6 decrease in rapeseed than in radish seeds, but sterilization was more acute in radish seeds after sterilization at 1.5 atm (Fig. 2(a)). Additionally, lower inositol phosphates were detected (Fig. 2(b)): IP-5 in pasteurised rapeseed sprouts, and IP-5 and also IP-4 in the same sprouts sterilised under both conditions (1 and 1.5 atm). In the case of radish sprouts, both pasteurised and sterilised, IP-3 was also identified besides the above-mentioned compounds (Fig. 2(b)).

The degradation of IP-6 to a level of ca. 50% after 4 days of germination and subsequent decrease below 10%, as a result of the thermal treatments applied, was found to increase the bioavailability of trace elements, as well as the digestibility of proteins and starch (Honke et al., 1998). On the other hand, a low concentration of IP-6 in germinated and thermally treated sprouts relates to the loss of a compound with highly valuable chelating properties (Graf & Eaton, 1990). However, the appearance of lower forms of inositol phosphates in sprouts after thermal treatment could have beneficial effects since they have been assigned a significant biological role in intracellular signal transduction systems as the second messenger (Streb, Irvine, Berridge, & Schultz, 1983).

Table 1 shows the effect of germination and heat treatments on the trypsin inhibitor activity of rapeseeds and radish sprouts. Raw seeds show a very low actvity of TIA (1.17 and 0.98 TlU/mg d.m., respectively). Germination conducted for 4 days caused a slight decrease in TIA: 18% in rapeseed and 4% in radish sprouts. Thermal treatments used for preservation completely elimi-

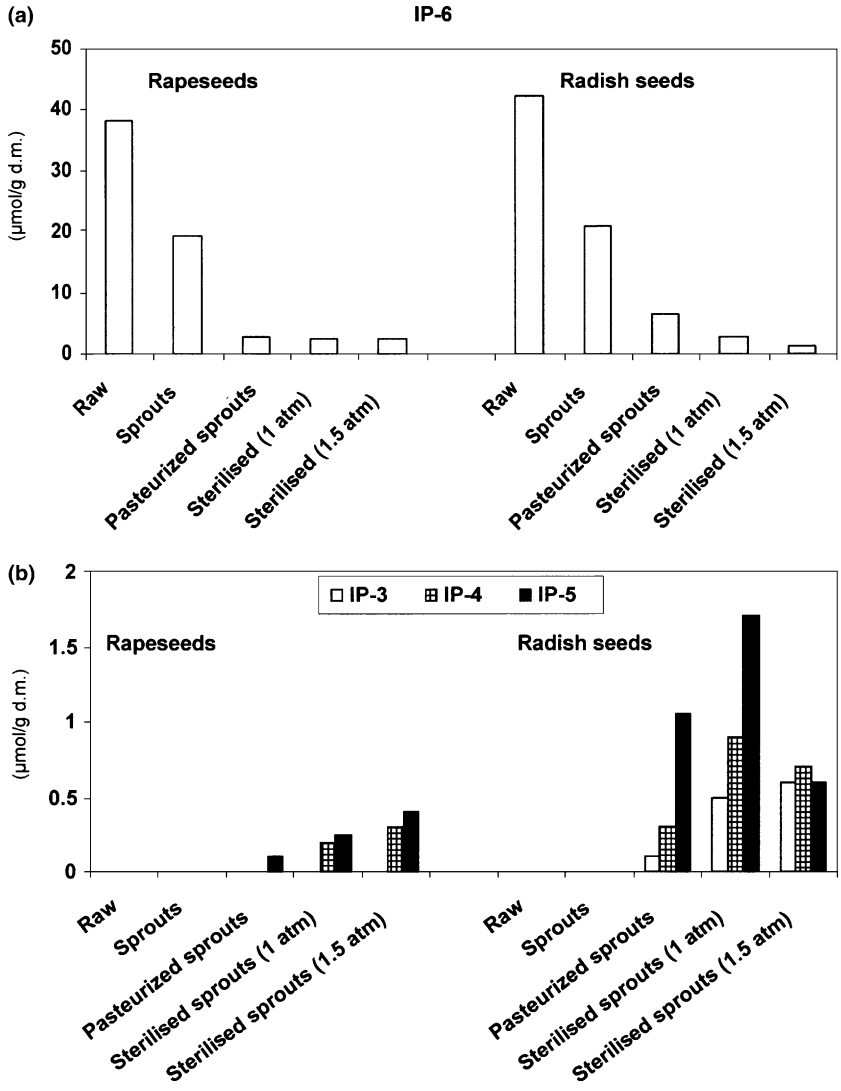

Fig. 2. Effect of heat treatment on the inositol-phosphate content of germinated cruciferous seeds.

Table 1
Effect of germination and heat treatment on the trypsin inhibitor activity of cruciferous seeds

Cruciferous seeds	Trypsin inhibitor units (TIU/mg d.m.)
Raw rapeseeds	1.17 ± 0.08^{b}
Rapeseed sprouts	0.95 ± 0.04^{b}
Pasteurized (95 °C/30 min) rapeseed sprouts	ND[a]
Sterilized (1 atm/30 min) rapeseed sprouts	ND[a]
Sterilized (1.5 atm/30 min) rapeseed sprouts	ND[a]
Raw radish seeds	0.94 ± 0.02^{b}
Radish sprouts	0.93 ± 0.02^{b}
Pasteurized (95 °C/30 min) radish sprouts	ND[a]
Sterilized (1 atm/30 min) radish sprouts	ND[a]
Sterilized (1.5 atm/30 min) radish sprouts	ND[a]

Mean value ± standard deviation of three determinations. The same superscript in the same column for each cruciferous seed means no significant difference ($P \leqslant 0.05$); ND, not detected.

nated TIA. No information has been found on the effect of germination on TIA in Cruciferae seeds. In legumes, TIA was modified to a different extent, depending on the type of seed and the germination conditions (Frias, Vidal-Valverde, Sotomayor, Díaz-Pollan, & Urbano, 2000; Frias, Díaz-Pollán et al., 1995; Vidal-Valverde et al., 2002). Trypsin inhibitors are heat-labile compounds and this fact justifies their removal after pasteurization and sterilization.

4. Conclusions

Germination reduced the content of IP-6 in the 4 examined seeds. After 4 days of germination, when sprouts were ready-to-eat, reduction in IP-6 reached ca. 50% and next ca. 40% reduction occurred after thermal treatment. Pasteurization and sterilization, besides a sharp reduction in IP-6, caused the creation of lower forms of inositol phosphates. The activity of trypsin inhibitors during 4 days of germination changed slightly and disappeared completely after thermal treatment.

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References

Bannerjee, S., Rohatgi, K., & Lahin, S. (1954). Pantothenic acid, folic acid, biotin and niacine contents of germinated pulses. *Food Research, 19*, 134–139.

Bewley, J. D., & Black, M. (1982). *Physiology and biochemistry of seeds in relation to germination* (Vol. 1 and 2). Berlin-Heidelberg, NY: Springer Verlag.

Birk, Y. (1994). Protein proteinase inhibitors in food. *Proceedings of the international Euro food to IV conference: Bioactive substances in food of plan origin, 22–24 September, Olsztyn, Poland* (Vol. 1, pp. 202–213). .

Cadenas, E., & Packer, L. (2002). *Handbook of antioxidants*. New York: Marcel Dekker.

Finley, P. L. (1978). Potential for the use of germinated wheat and soybean in human nutrition. *Journal of Food Science, 43*, 681–701.

Frias, J., Vidal-Valverde, C., Sotomayor, C., Díaz-Pollan, C., & Urbano, G. (2000). Influence of processing on available carbohydrate content and antinutritional factors of chick peas. *European Food Research and Technology, 210*, 340–345.

Frías, J., Díaz-Pollán, C., Hedley, C. L., & Vidal-Valverde, C. (1995). Evolution of trypsin inhibitor activity during germination of lentils. *Journal of Agricultural and Food Chemistry, 43*, 2231–2234.

Frías, J., Díaz-Pollán, C., Hedley, C. L., & Vidal-Valverde, C. (1996). Evolution and kinetics of nonosacharides, disacharides and α-galactosides during germination of lentils. *Zeitschrift fuer Lebensmittel-Untersuchung und-Forschung A, 202*, 35–39.

Graf, E., & Eaton, J. W. (1990). Antioxidant functions of phytic acid. *Free Radical Biology and Medicine, 8*, 61–69.

Graf, E., & Eaton, J. W. (1993). Suppression of colon cancer by dietary phytic acid. *Nutrition and Cancer, 19*, 11–19.

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 fuer Lebensmittel-Untersuchung und-Forschung A, 206*, 279–283.

Kakade, M. L., Rackis, J. J., McGhee, J. E., & Puski, G. (1974). Determination of trypsin inhibitor activity of soy bean products: a collaborative analysis of an improved procedure. *Cereal Chemistry, 51*, 376–382.

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 rapeseeds for sprouts production in human nutrition. *Oilseed Crops, XXIII*(1), 165–173.

Kuo, T. H., & Van Middlesworth, J. F. (1988). Content of raffinose oligosaccharides and sucrose in various plants. *Journal of Agricultural and Food Chemistry, 36*, 32–39.

Liener, I. E., & Kakade, M. L. (1978). Protease inhibitors. In I. E. Liener (Ed.), *Toxic constituents of plant foodstuffs* (pp. 7–71). New York: Academic Press.

Lönnerdal, B., Sandberg, A. S., Sandström, B., & Kunz, C. (1989). Inhibitory effects of phytic acid and other inositol phosphates on zinc and calcium absorption in suckling rats. *Journal of Nutrition, 119*, 211–214.

Price, T. V. (1988). Seed sprouts for human consumption – a review. *Canadian Institute of Food Science and Technology Journal, 21*(1), 57–65.

Prodanov, M., Sierra, I., & Vidal-Valverde, C. (1997). Effect of germination on the thiamine, riboflavin and niacin contents in legumes. *Zeitschrift für Lebensmittel-Untersuchung und-Forschung A, 205*, 48–52.

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

Reddy, N. R., Pierson, M. D., Sathe, S. H., & Salunkhe, O. K. (1989). *Phytates in cereals and legumes. Influence of processing technologies on phytate*. Boca Raton: CRC.

Roberfroid, M. B. (2000). Defining functional foods. In G. R. Gibson & C. M. Williams (Eds.), *Functional foods*. Cambridge, England: CRC Press, Woodhead Publishing Limited.

Sandberg, A. S., & Ahderinne, R. (1986). HPLC method for determination of inositol tri-, tetra-, penta-, and hexaphosphates in food and intestinal contents. *Journal of Food Science, 51*, 547–550.

Sandberg, A. S., Carlsson, N. G., & Svanberg, U. (1989). Effects of inositol tri-, tetra-, penta-, and hexaphosphates on in vitro estimation of iron availability. *Journal of Food Science, 54*, 159–161.

Shamsuddin, A. M. (1995). Inositol phosphates have novel anticancer function. *Journal of Nutrition, 125*, 725–732.

Streb, H., Irvine, R. F., Berridge, M. J., & Schultz, I. (1983). Release of Ca^{2+} from non mitochondrial intracellular store in pancreatic acinar cells by Ins(1,4,5)P_3. *Nature, 306*, 67–69.

Takaya, Y., Kondo, Y., Furukawa, T., & Niwa, M. (2003). Antioxidant constituents of radish sprout (Kaiware-daikon), *Raphanus sativus* L. *Journal of Agricultural and Food Chemistry, 51*, 8061–8066.

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

Valdebouze, P., Bergeron, E., Gaborit, T., & Delort-Laval, J. (1980). Content and distribution of trypsin inhibitors and haemaglutinins in some legume seeds. *Canadian Journal of Plant Sciences, 60*, 695–701.

Vidal-Valverde, C., Frias, J., Sierra, I., Blazquez, I., Fernand Lambein, F., & Kuo, Y. H. (2002). New functional legume food by germination. Effect on the nutritive value of beans, lentils and peas. *European Food Research and Technology, 215*, 472–477.

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

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

Zieliński, H., Piskuła, M. K., Buciński, A., & Kozłowska, K. (2003a). Total antioxidant capacity and its components of Cruciferae seed sprouts. In *European conference on new functional ingredients and foods: Safety health and convenience, 9–11 April 2003, Copenhagen, Denmark* (Abstrat. book: P2-B23).

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

Zieliński, H., Piskuła, M. K., Buciński, A., & Kozłowska, H. (2003b). Antioxidant status and bioactive compounds of ready-to-eat rapeseed sprouts. In *Proceedings of the 11th international rapeseed congress, The Royal Veterinary and Agricultural University, Copenhagen, Denmark, 6–10 July 2003* (pp. 599–601).