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Changes in Some Chemical Components During Germination of Broccoli Seeds

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Abstract

Seeds germination to obtain sprouts is a technological method used for many years. During this biological process metabolic enzymes are activated and utilization or synthesis of wide range of chemical compounds occurs in seeds. Cruciferous plants seeds are especially valuable for this purpose because of glucosinolates abundance, the amount of which during germination even increases. The aim of this study was to determine the influence of germination process of broccoli (*Brassica oleracea* L. var. *botrytis* Plenck) seeds on their chemical composition related to nutritional value, including amino acids content. The seeds were germinated for 24, 72 and 120 h in the dark, at temperature of 25°C. The biggest changes in chemical composition were observed after 72 h of germination. During this process the amount of crude proteins increased, as well as amino acids content. Total amount of conjugated amino acids in non-germinated broccoli seeds was on the level of 181 g kg^{-1} dm and increased after 72 h of germination to 217 g kg^{-1} dm, while sudden decrease was observed after 120 h of germination, to the level of 177 g kg^{-1} dm. Prevailing essential amino acids in non-germinated and germinated seeds were leucine and arginine, while non-essential – glutamic acid.

Keywords: amino acids, broccoli, germination, sprouts, chemical content

Introduction

Interest in the healthy diet has increased during recent years. Raw agricultural products provide not only essential nutrients necessary for human body, but also a large range of biologically active compounds, which promote health and prevent from civilization diseases. Seeds germination for a food ('vegetable sprouts') is quite old processing method, used already for many centuries in far-East countries. Nowadays, the sprouts became also a fashionable food ('so called 'healthy food') in western countries because of low caloric value, high biological activity, reduced anti-nutritional components content and improved digestibility (Augustin and Klein, 1989; Ghorpade and Kadam, 1989; Frıas *et al.* 2007).

Cruciferae vegetables contain large amounts of vitamins, minerals and a special group of phytochemicals called glucosinolates, which show chemoprotective effect against cancer (Moreno *et al.,* 2006). Concentration of potentially beneficial glucosinolates has been found to be greater in seed sprouts, than in fully grown plants (Fahey *et al.*, 1997). Generally, their concentrations in the sprouts reaches 10% of dry matter, whereas in leaves, stems and roots the concentration is 10-times lower (Songsak and Lockwood, 2002). Therefore, it can be concluded that an increased consumption of broccoli sprouts may stimulate the natural defence system against cancer even more than consumption of broccoli heads (Rychlik and Sieghard, 2008). Glucosinolates are responsible for the unique taste of many foods and in the case of broccoli enhanced levels of glucoraphanin impart a strong bitter taste of this vegetable (Songsak and Lockwood, 2002).

Nutritional quality of proteins, which is related to their ability to support the body growth, depends on the content of essential amino acids. Germination is a biotechnological process, in which metabolic enzymes, such as proteinases, are activated. As a result of this process, some amino acids and peptides can be released, and the synthesis or utilization of others, to form new proteins, can occur. As a consequence, the nutritional quality of proteins can be enhanced, which is why the germination is suggested as a technological procedure for improving the nutritional quality of legumes and other seeds (Gulewicz *et al.,* 2008). Amino acid composition of food affects taste and flavor, as well. A number of amino acids has a distinctively bitter taste (eg. tyrosine, arginine, leucine, valine, methionine and histidine).

Many studies have been carried out to determine the effect of cruciferous seeds germination process on some biologically active compounds, but very few data gives information about changes in amino acids, which can occur. The objective of this study was to establish the influence of germination process of broccoli seeds on chemical composition related to their nutritional value, including amino acids content.

Materials and methods

Broccoli (*Brassica oleracea* L. var. *botrytis* Plenck) seeds were purchased in a shop selling 'healthy food', in Lithuania. Seeds were germinated in 'Bio-Natura' sprouters (plate capacity – 1L, diameter – 20 cm) in the dark, at the temperature of 25°C, for the period up to 120 h. For sprouting, 250 g of the seeds were washed and imbibed for 12 h in water (ratio 1:4). The germination was performed in triplicate. Germinated seeds were dried in an electric oven at 65°C for 24 h and milled. Chemical analyses were performed in the raw (non-germinated) seeds and the ones germinated for 24, 72 and 120 h.

Dry matter content of the dry and germinated seeds was determined gravimetrically, by drying them to the constant weight at 105°C.

Crude proteins, crude fats, crude ash, crude fiber amounts were determined according to the method described by Nauman and Bassler (1993).

The amount of nitrogen free extracts was calculated according to the principle of difference: amounts of crude proteins, crude fats, crude fiber and crude ash took from 100 (Oloyo, 2004).

Amino acids were separated by the method of ion-exchange chromatography and detected photometrically by measuring light absorbance at 570 nm with automatic analyzer of amino acids Mikrotechna AAA 339, using glass column (∅ 0.37×45 cm), filled with ionite Ostion LGANB. Hydrolysis of the sample was performed in the presence of 6 M HCl at 105°C for 24 h. The essential amino acids: threonine, valine, methionine, isoleucine, leucine, phenylalanine, histidine, lysine, arginine and non-essential amino acids: aspartic acid, serine, glutamic acid, glycine, alanine, tyrosine were determined (Technical regulation of amino acids ..., 2003).

Energetic value was calculated according to Osborne and Voogt (1978), multiplying the percentage of crude protein, crude fat and nitrogen free extracts by the factor of 4, 9 and 4, respectively.

Statistical analysis

The data obtained were statistically analyzed with ANOVA, and Tukey's HSD test was performed, using SigmaStat 2.03 software. Significant differences between means were determined at $P < 0.05$. The data are reported as means and ± standard deviation (SD).

Results and discussions

Dry matter losses are inevitable in germinating seeds because of imbibing and other physiological processes that take place during the germination. One of the basic tasks in seeds germination technology is minimizing dry matter losses, since the higher dry matter amount in the product – the higher its nutritional value. Dry matter losses during seeds germination period depended on germination time. The highest loss was observed during the first 24 h of germination (Fig. 1). During 120 h of germination, the loss of dry matter in broccoli seeds reached about 80%.

In order to estimate the dependence between dry matter losses and seeds germination time, a correlation coef-

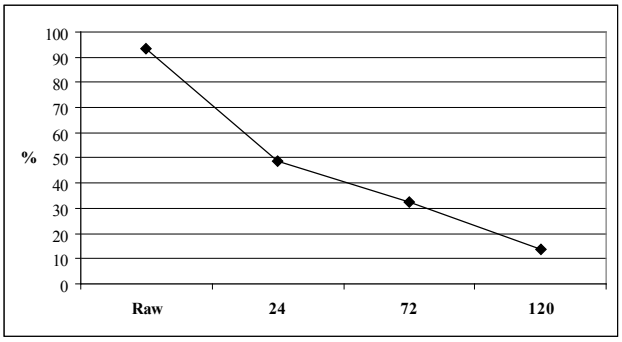

Fig. 1. Dry matter changes in germinating broccoli seeds

ficient *r* was calculated. It was equal to – 0.854, at the probability level 95%. The main cause of dramatic dry matter losses during the first 24 hours may be seeds intensive imbibing at the beginning of the germination. It was found that during rape seeds germination (*Brassica napus* L. var. *oleifera*) the most intensive dry matter losses were observed during the first four days of germination (Zielinski *et al*., 2006).

The amount of nitrogen free extract and crude fats decreased during the germination period (Fig.2). At the same time, the amounts of crude proteins in seeds significantly increased during germination, compared with the raw seeds. At the end of germination process crude protein content stabilized. The amounts of crude fiber in the germinating seeds significantly increased up to 72 h of germination, whereas the amount of crude ash in seeds was stable.

In the germination process changes in the energetic values of the seeds were quite high, and there was decreasing from 1819 kJ 100 g–1 in the case of raw seeds to 1526 kJ 100 g–1 in germinated seeds (Fig. 3). That may be related to crude fat amount decreasing during seeds germination (almost by 29%, when comparing raw and 120 h germinating seeds), since 1 g of fats provides 37.68 kJ, i.e. pro-

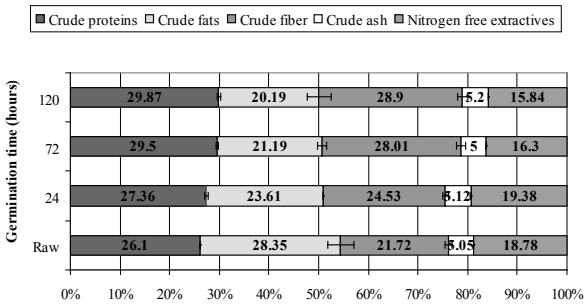

Fig. 2. Chemical composition changes during broccoli seeds germination

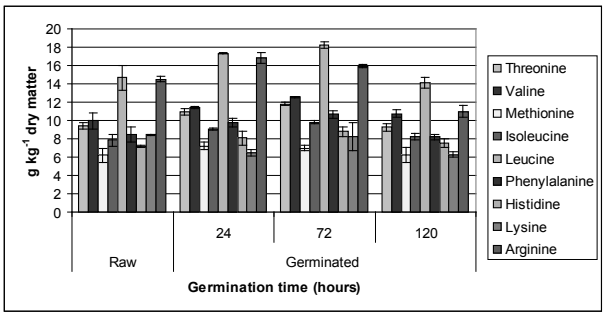

Fig. 4. Essential amino acids changes during broccoli seeds germination

vides almost twice more energy than other components of the seeds.

The dominating essential amino acids in broccoli seeds were leucine and arginine, which are related to bitter taste of product (Fig. 4). Significant increase of leucine was observed after 72 h of germination. In the case of methionine, which is essential for cysteine synthesis during broccoli germination, we found no significant changes. Amounts of phenylalanine and leucine decreased after 120 h on about 23% and 18%, respectively. Germination resulted in increase of isoleucine, valine and histidine amounts. Definitely almost all essential amino acids amounts increased in germinating seeds till 72 h of germination, while after 120 h slight decrease in their amount was observed (Fig. 4).

The prevailing non-essential amino acid was glutamic acid, while tyrosine was found in the lowest amount in the seeds (Fig. 5). Amounts of aspartic acid, alanine, serine and tyrosine increased after 72 h of germination, compar-

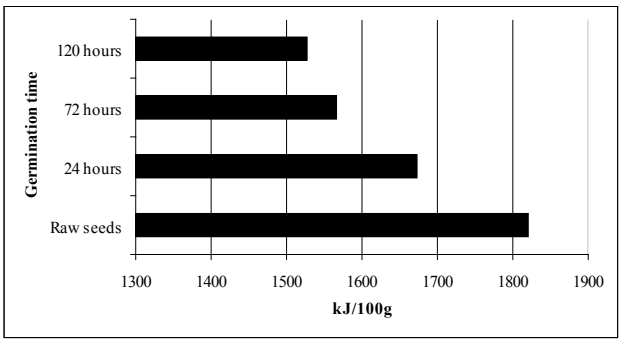

Fig. 3. Energetic value changes of germinated broccoli seeds

ing with raw seeds, of 5.15; 4.93; 2.34 and 1.31 g kg^{-1} dm, respectively. Prolonged germination process (till 120 h) caused decrease of essential and non-essential amino acids content. The total amino acids amount was the highest in 72 h of germination process (217.6 g kg^{-1} dm) and the least in 120 h (176.9 g kg^{-1} dm) (Fig. 4 and Fig. 5). Crude proteins content also increased up to 72 h of germination, while after 120 h it did not change significantly, comparing with the content at 72 h of germination (Fig. 3).

During the first 72 h of germination changes in the

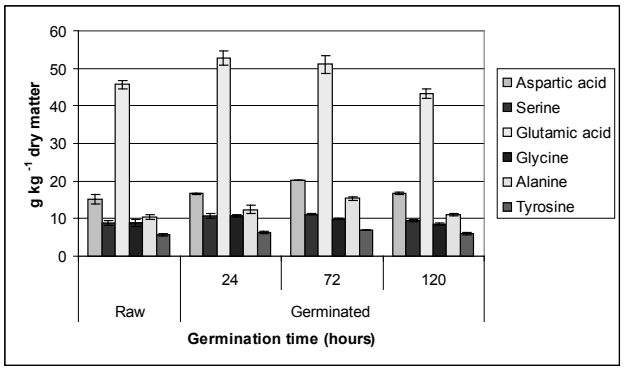

Fig. 5. Non-essential amino acids changes during broccoli seeds germination

amino acid content in broccoli seeds could be related to protein hydrolysis, synthesis, and re-arrangement. According to Rodrıguez *et al.* (2008), seeds germination involves mobilization of the protein reserves in cotyledons, coupled with the synthesis of new proteins, necessary for sprout's growth. Furthermore, the amino acids produced by hydrolysis of the protein reserves are not used solely in synthesizing new components, but may also be used as an energy source, especially in the early stages of the germination (Chen *et al.,* 1975). One of the indicators of protein quality is the ratio of essential to total amino acids amounts. The essential to total amino acids ratio for broccoli seeds was higher than reported in literature (33.9%) (FAO/WHO, 1991). In the case of raw seeds this ratio was equal to 47.8%, in seeds germinated for 24 h – 46.9%, for 72 h – 47.3% and for 120 h – 46.1%.

Conclusions

During seeds germination dry matter content decreases and the most dramatic changes occur during first 24 h of germination process. It can be caused by water absorption and intensive respiration process in seeds. Significant

changes in amino acids content in broccoli seeds are observed up to 72 h of the germination. Total amount of essential and non-essential amino acids increases during the first 72 h of germination, while after that period slightly decreases. Amino acids content increase in germinating seeds corresponds with crude protein content increase. As a result of crude fats losses, energetic value of germinating broccoli seeds decreases. It can also be concluded that the highest nutritional value shows broccoli seeds germinated for 72 h.

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