Digestive Enzyme Inhibitors from Grains as Potential Components of Nutraceuticals

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Summary Inhibitor activity against digestive enzymes, such as α-amylase from human saliva and porcine pancreas and trypsin from bovine pancreas, of three cereal grains species were studied as potential components of nutraceuticals strengthening diabetes and obesity treatment. Significant differences were demonstrated: the highest antitryptic activity was found in the grain of the rye varieties studied, whereas the grain of the wheat varieties had significantly higher ability to inhibit α-amylases from human saliva and porcine pancreas. Additionally, seeds of Puma, one of the studied wheat varieties, demonstrated especially low antitryptic activity. Such a beneficial arrangement of inhibitors, i.e. high level of inhibitors of α-amylase from human saliva and porcine pancreas and simultaneously low level of trypsin inhibitors from bovine pancreas, indicate the possibility of the application of seeds with such properties to the preparation of nutraceuticals for people with obesity or suffering from diabetes.

Key Words wheat, rye, triticale, amylase inhibitors, trypsin inhibitors

Numerous studies have demonstrated that cereal grains contain variable numbers of proteins with characteristics of digestive enzyme inhibitors of mammals, insects, and bacteria (1–7). These proteins for many years were recognized only as undesirable, anti-nutritional substances: they decrease the availability of nutritional components (6–11) and moreover are listed among protein fractions causing food allergies (12, 13). However, in recent years, more and more studies have demonstrated that these proteins can be utilized as components of therapeutic and health-related products under some conditions (8, 14, 15). Nutritional studies concerning the application of cereal grain diets rich in inhibitors of α-amylases proved that their biological activity significantly influences the metabolism of starch of the studied organisms. The proteins consumed in food are resistant to digestion by pepsin and they are transported in their unchanged form to the duodenum, where they inactivate porcine pancreas α-amylase (16). Inhibitors of α-amylases can play a significant function in diet therapy for metabolic syndrome, because they hinder absorption of saccharides into the alimentary tract, which lowers the glycemic index of consumable food (15, 17). They also play a role as substances lowering insulin-resistance. These facts indicate that these substances are able to modify the process of starch digestion as well as to lower postprandial hyperglycemia (8, 15).

In cereal grains, proteinaceous substances with anti-proteolytic activity, mainly antitryptic, are present as well (9, 10). The mechanism of action of these inhibitors relies on forming a stable proteolytic enzyme-inhibitor complex, which is formed as a result of the reaction of free amine groups of trypsin and carboxyl groups of the inhibitor (18). Formed complexes are dissociated to a small degree. The availability of food protein is limited and therefore there is a loss of the essential amino acids, mainly the exogenous methionine. Additionally, the organism is subjected to a loss of endogenous digestive enzyme activities (19).

Trypsin inhibitors are thermolabile, and partially lose their activity as a result of heat treatment, which occurs during cereal grain processing (e.g. baking) (9, 19, 20). It was observed that the most abundant group of oat trypsin inhibitors were inactivated during 10-min incubation at a temperature of 80°C or higher and pHs of 3.3 or lower. However, some of them were present even after 30 min of boiling (21). These results suggest that only long-term hydrothermal processing of cereal grain fully limits the activity of protease inhibitors.

Because cereal inhibitors of amylases and trypsin are resistant to digestion and some technological processes used in food and nutraceutical processing, they very easily will permeate in the unmodified form into an organism (2, 9, 15, 16). Comparison of the inhibitory activities of three cereal grain species as a potential component of special purpose foods, supporting the treatment of diabetes and obesity, was the aim of this work.
Materials and Methods

The study was carried out on two varieties of triticale (Presto and Vero), rye (Motto and Amilo), and wheat (Lama and Puma), each harvested during 4 consecutive years at the Breeding Station DANKO in Choryń, Poland. The grain was cultivated in the same experimental plots under natural agro-technical conditions.

Extraction of inhibitors from cereal grains was performed according to the method described by Warchalewski et al. (22). The extraction was carried out with 3 independent replications. Determination of inhibitory activities against exogenous enzymes of α-amylases from human saliva (Sigma A1031-77 UAA/mg protein) and porcine pancreas (Sigma 10080-675 UAA/mg protein) was done according to the Bernfeld colorimetric method (23). Determination of inhibitory activity against trypsin from bovine pancreas (Sigma T4665-129 UAP/mg protein) was performed according to the Nomoto and the Narahashi method (24). Inhibitory activities of the studied extracts were expressed in units, as a total activity amount per 100 g of dry matter.

All samples were analyzed fivefold.

Obtained results were subjected to statistical analysis. Two-way analysis of variance (ANOVA) was applied to study significant differences in activities of inhibition against α-amylase and trypsin, between varieties, and cereal grains species and years of vegetation. In addition, interaction between varieties and years of vegetation was analyzed. The post-hoc Tukey’s test was used to analyze differences and determine homogenous groups. The Shapiro-Wilk test was used for the assumption of normality, and the Levene test for homogeneity of variance. Data were analyzed with the use of Statistica 8.0 software. All tests were considered significant at α=0.05.

Results and Discussion

The studies were carried out on varieties of three cereal grain species grown for 4 consecutive vegetation years. In Poland climate conditions differ mainly in monthly quantity of rainfall and insolation during vegetation period of cereals. It influences their biological, physical, biochemical and technological properties (25). The measurement results of inhibitory activities against digestive enzymes of cereal grains studied in individual years are shown in Figs. 1–3.

The studied grains had differentiated inhibitory activity against mammalian digestive enzymes among studied cereal grains species and among individual har-
vesting years.

Distinguishable differences between studied cereal grain species and individual years of harvest were proved based on two-way analysis of variance of measurement results of inhibitory activities of the grains against individual digestive enzymes. Interrelationship between the year of harvest and cereal species is very important. Therefore, the inhibitory activities against α-amylase from human saliva and from hog pancreas and trypsin from bovine pancreas were different in individual years in grains of individual cereal species. Such differences are not surprising, and due to this fact, this kind of experiment should not be conducted only for cereals from one crop. The considerable changes in inhibitor activity in dependency on harvest year and variety was also observed by other authors (25). Obtained results demonstrated that the seeds of wheat varieties in comparison to the remaining studied cereal grains species had significantly higher ability to inhibit α-amylase from human saliva (depending on the cultivation year, at least 40% higher than triticale and at least 50% higher than rye) (Fig. 1). Seeds of both wheat varieties also showed the highest total inhibitory activity against α-amylase from hog pancreas. This activity was at least 30% higher in comparison to rye seeds (third-year crop) and at least 10% higher in relation to triticale (second-year crop) (Fig. 2). Among examined cereal grains, both rye variety seeds had the highest antitryptic activity, while the lowest was observed for wheat variety Puma in every year. In the first crop year, the Puma seeds had up to 20 times lower antitryptic activity than rye seed Amilo variety and up to 14 times lower than triticale Presto variety.

Antitryptic activity differed significantly among varieties of wheat. In the first studied harvest year, wheat variety Lama had more than 10-fold higher activity in relation to wheat variety Puma (Fig. 3). Due to various activities of inhibitors, grains used in food processing can influence consumers in different ways. A grain with high antitryptic activity, for example the rye varieties studied, can limit absorption of food proteins (11, 26), which decreases its nutritional usefulness. Hyperplasia or hypertrophy of the pancreas as well as an increase in the risk of pancreatic cancers are the far-reaching consequences of an excessive amount of trypsin inhibitors in food rations (16, 26, 27). Simultaneously, the seeds of cereals with high inhibitory activity against amylase can cause modification in the process of starch digestion, influencing the lowering of postprandial hyperglycemia (8, 15, 17). This finding has significant meaning for patients suffering from diabetes.

Among cereal grains studied, the two wheat varieties had the highest ability to inhibit mammalian α-amylases in all the studied years. Additionally, seeds of wheat variety Puma in comparison to the remaining studied cereal grain varieties, indicated small, many times lower, antitryptic activity. This is a variety feature of the Puma grains, because antitryptic activity of grains of wheat variety Lama was repeatedly higher in every studied year, and even 10 times higher in the first year of the study. The beneficial system of inhibitors in the seeds of wheat variety Puma, such as the high level of inhibitory activity against α-amylases from human saliva and porcine pancreas and low level of inhibitory activity against bovine pancreas trypsin, indicates the possibility of their application for the preparation of special purpose foods. The well recognized thermostability of α-amylase inhibitors (3) and suggested partial thermolability of trypsin inhibitors (25) create wide opportunities for the use of grains with such properties to produce various types of nutraceuticals. Some authors detected the presence of active amylase inhibitors in wheat flour baked products (28).

Inhibitors of α-amylases contained in such products can have an influence on effective inhibition of α-amylase from the pancreas that is secreted into the duodenum, which can be useful in diabetes and obesity treatment (29). Animal feeding tests with a diet fortified by wheat rich in amylase inhibitors and poor in trypsin inhibitors, such as the Puma variety, is necessary for confirmation of its usefulness in nutraceuticals.

Such a beneficial set of inhibitors is a variety trait; therefore, control of seeds intended for special-purpose food production in the context of the presence of inhibitors is reasonable.
REFERENCES

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