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THE EFFECT OF PROTEOLYTIC ACTIVITY ON THE TECHNOLOGICAL VALUE OF WHEAT FLOUR FROM PRE-HARVEST SPROUTED GRAIN

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Abstract. Investigations were conducted on the level of the overall proteolytic activity in flour fractions as well as fine and bruise bran, obtained from four varieties of wheat ('Zyta', 'Pegassus', 'Sukces', 'Tonacja'), subjected to pre-harvest sprouting. Moreover, an analysis was conducted on the effect of pre-harvest sprouting on the functional properties of flour, determining the physical properties of gluten and dough. The analyses included a determination of crude protein, non-protein nitrogen, wet gluten, proteolytic activity and the rheologic properties of dough. The studies ended with a trial baking, with vitamin C and vital gluten added as improvers to the flour from pre-harvest sprouted grain. In all the milling fractions the overall proteolytic activity increased as result of sprouting, the highest increase being recorded for variety 'Tonacja'. Simultaneously, in all the fractions tested an increased level of non-protein nitrogen was observed. Flour obtained from pre-harvest sprouted grain was characterised by an increased water holding capacity and the dough by poorer rheologic properties. Bread obtained from flour from pre-harvest sprouted grain was of insufficient quality. The use of improvers (vital gluten and vitamin C) as a rule resulted in favourable palatability and physico-chemical changes in bread.

Key words: wheat, pre-harvest sprouting, proteolitytic activity, baking value

INTRODUCTION

Wheat flour contains various enzymes which affect its technological value – among others amylases, proteinases, lipases, lypoxygenases, polyphenol oxidases and peroxidases. Of the enzymes listed, the following hydrolases play a key role in the forming of the baking value of flour: proteinases which degrade proteins to small molecule compounds, amylases, for which starch is the substrate and lipases, separating higher fatty acids from triacylogliceroles. Despite the fact that those enzymes during a correct storage of grain and flour, remain inactive or, as in the case of lipases, active to only a lim-

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ited degree, under the influence of water they catalyse specific reactions and thus directly affect the functional properties of flour [Rani et al. 2001].

In earlier works the same authors [Dojczew et al. 2004, 2005] analysed the level of activity of chosen hydrolases (amylases and proteinases) in flour from wheat, rye and Triticale, obtained from pre-harvest sprouted grain. The results reported in the present work are a continuation of the previous studies and result from an analysis of the changes in the proteolytic activity in individual milling fractions, obtained from four varieties of pre-harvest sprouted winter wheat. Samples used for the analyses differed in the content of crude protein, gluten and the quality class.

The present work aimed at determining the overall proteolytic activity in the milling fractions of wheat differing in quality, earlier subjected to pre-harvest sprouting as well as examining the effect of his indicator on the baking value of flour.

METHODS

The studies were conducted on four varieties of wheat: 'Zyta', 'Pegassus', 'Sukces' and 'Tonacja', obtained from the Experimental Farm in Chylice. The grain was subjected to pre-harvest sprouting for four days in controlled, laboratory conditions. Healthy, dried and pre-harvest sprouted grain was ground in a Quadrumat Senior laboratory mill. The flour from pre-harvest sprouted and not sprouted grain was analysed for protein and non-protein nitrogen according to the method by Kjeldahl, and for wet gluten. Moreover, a farinographic analysis was conducted using Brabender's farinograph. The interpretation of farinogrames was performed by the AACC method [AACC... 1972]. The overall proteolytic activity was determined by the method by Fritz [Fritz et al. 1974, Dojczew et al. 2004] in fine bran, bruise bran and flour. The studies ended with a test laboratory baking, performed by the direct method for wheat bread, recommended by the Bakery Institute in Berlin. The flour from pre-harvest sprouted grain was supplemented by two improving preparations: 0.1% ascorbic acid and 3% vital gluten (in relation to the weight of flour).

The results obtained were subjected to a single factor analysis of variance Anova, using the computer software Statgraphics Plus 4.1. The significance of differences between mean values for $\alpha = 0.05$, depending on the effect of different factors, was verified by the LSD test.

RESULTS AND DISCUSSION

The level of the overall proteolytic activity in caryopsis indicates their physiological condition. The content of non-protein nitrogen is one of the indicators of an increased activity of proteases. Figure 1 presents the content of non-protein nitrogen in model samples as well as in flour and bran from pre-harvest sprouted grains. The share of non-protein nitrogen in model samples differed between varieties and between individual milling fractions. The highest value for this indicator was observed in bruise bran, which may arise from the fact that fragments of the aleuron layer and plumule, rich in free amino acids and small-molecule peptides, have passed to this fraction. As shown in

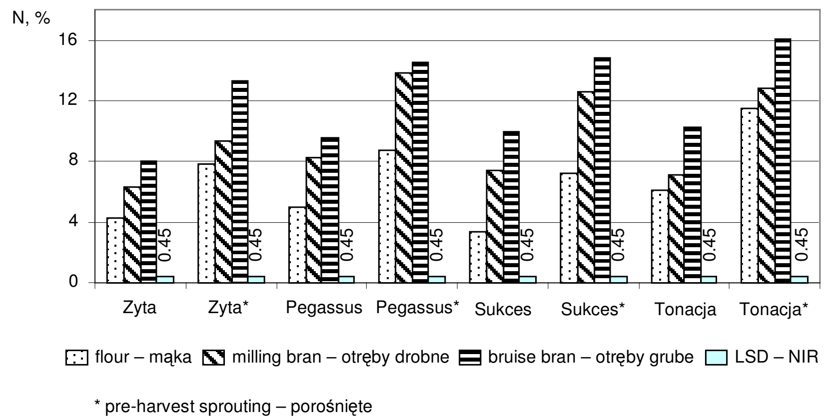

Fig. 1. The effect of the pre-harvest sprouting of grains on the non-protein nitrogen content in the particular milling fractions
Rys. 1. Wpływ porostu ziarna na udział azotu niebiałkowego we frakcjach przemiałowych

earlier studies [Bielawski et al. 1995, Dojczew et al. 2003, 2005] sprouting resulted in an almost double increase of non-protein nitrogen in each of the varieties and fractions examined. The increased content non-protein nitrogen is, on one side, the result of the activation of proteases and on the other the result of a *de novo* biosynthesis of amino acids and peptides in the newly forming tissues of the shoot. The higher values obtained for non-protein nitrogen in both bran fractions than in the flour was caused by the location of proteolytic enzymes principally in the peripheral part of the caryopsis, what has been confirmed by the works of Bethke et al. [1989] and Dojczew et al. [2005]. Among the wheat varieties examined the greatest increase of non-protein nitrogen in the flour and bruise bran was observed for ‘Tonacja’.

The level of the overall proteolytic activity in individual milling fractions (Fig. 2) reflected the content of non-protein nitrogen. In model samples of flour and fine bran only small differences were observed between varieties, while the activities recorded for the ‘Tonacja’ variety were slightly higher. During pre-harvest sprouting of caryopsis proteases are one of the first groups of hydrolases that are subjected to expression [Bethke et al. 1998, Bleukx and Delcour 2000]. Thus, as result of the sprouting process the proteolytic enzymes were activated principally in the aleuron layer and the plumule, because the highest activity was shown in bruise bran of all varieties. The highest level of proteolytic activity in all milling fractions of the ‘Tonacja’ variety remains in agreement with the fact that they showed also the highest level of non-protein nitrogen.

The increased activity of proteases resulted in changes not only in the primary structure but also in the secondary structure of caryopsis proteins and other gluten proteins. The partial hydrolysis of gluten proteins lead to the destruction of some bindings stabilizing their conformation. The disturbance of the special arrangement consisted probably of eliminating also the β-helical structures which, according to Shewry et al. [2001] and Gianibelli et al. [2001], are principally responsible for the functional properties of gluten proteins.

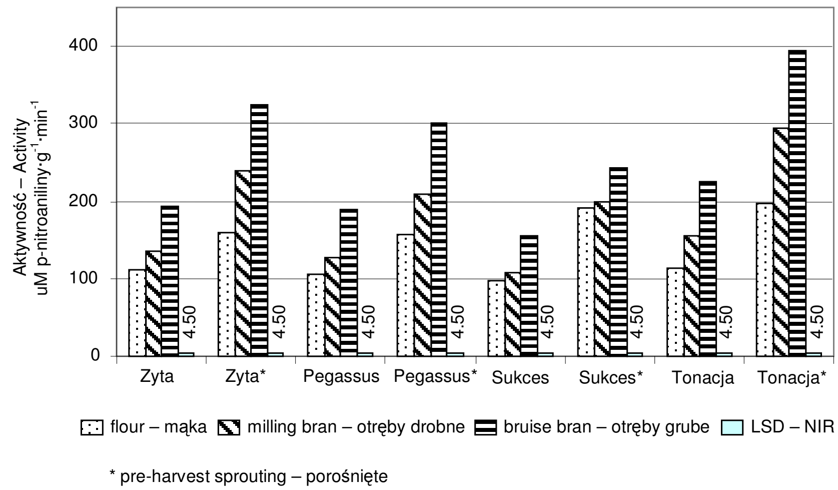

Fig. 2. The effect of the pre-harvest sprouting of grains on the proteolytic activity in the particular milling fractions
Rys. 2. Wpływ porostu ziarna na aktywność proteolityczną we frakcjach przemiałowych

The considerable changes in the quantity and quality of gluten in samples of pre-harvest sprouted grain were confirmed by farinographic analyses. The content of wet gluten (Table 1) in model samples remained in agreement with standards (PN-77/A--74041). The highest level of gluten was recorded for variety 'Zyta' and the lowest for variety 'Tonacja' and 'Sukces'. As effect of pre-harvest sprouting the quantity of gluten proteins was markedly decreased in each sample analysed and the differences observed were confirmed statistically. It proved impossible to isolate gluten from flour made from pre-harvest sprouted grain of the 'Tonacja' variety. The specially high activity of proteases in this sample resulted in a destruction of the gluten complex into low-molecule sub-units, easily moving to water solutions, together with soluble proteins.

Changes in the structure of gluten proteins as result of pre-harvest sprouting were reflected in rheologic tests of the dough. The dough obtained from flour from pre-harvest sprouted grain (Figs 3 and 4) was characterised by an unfavourable and statistically significant shortening of the time of development and stability. In the farinographic evaluation of flour another important quality trait is the softening. In all the samples of pre-harvest sprouted grain an increase in this indicator was observed, expressed by a decrease of the dough consistency. The poorer rheologic properties of dough caused by pre-harvest sprouting was most marked in relation of the 'Tonacja' variety.

The studies were ended by a laboratory baking, for which the flour from pre-harvest sprouted grains was supplemented with vital gluten and vitamin C as improving substances. Bread obtained from control flours was characterized by correct palatability properties: loaves were of adequate volume, showed a smooth, unbroken crust of a correct thickness and colour, the crumb was marked by a very good elasticity and uniform porosity, while the aroma and taste were typical of wheat bread. The physico-chemical properties of this bread were as follows: the volume of 100 g of bread, its efficiency, acidity and the crumb porosity were compliant with standard PN-92/A-74105.

Table 1. Analysis of quantity and quality of wet gluten
Tabela 1. Analiza ilości i jakości glutenu

Kind of cereal Gatunek zboża	Content of wet gluten Zawartość glutenu mokrego %	Gluten spreadability Rozpływalność glutenu mm
Zyta	40.5a	7a
Zyta*	36.5b	17b
Pegassus	34.5a	9a
Pegassus*	21.6b	18b
Sukces	34.0a	9a
Sukces*	28.7b	16b
Tonacja	34.0	9
Tonacja*	X	X

*Flour of pre-harvest sprouting grain.
a, b – means in the same column with different superscripts are significantly different at α = 0.05.
X – no isolate gluten.
*Mąka z ziarna porośniętego.
a, b – wartości oznaczone różnymi literami różnią się statystycznie istotnie na poziomie α = 0,05.
X – nie wymyto glutenu.

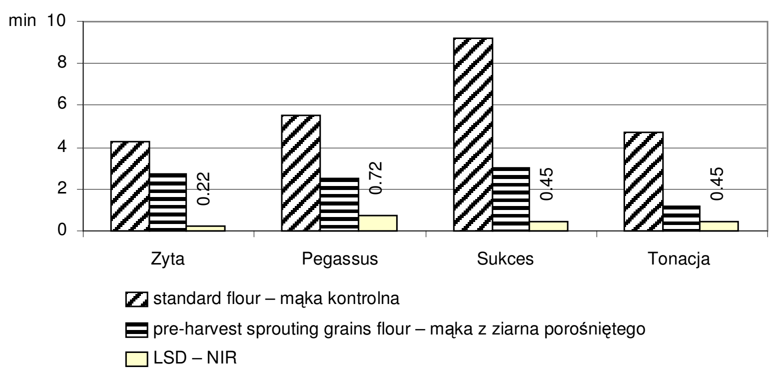

Fig. 3. The effect of the pre-harvest sprouting of grains on the development time of dough
Rys. 3. Wpływ porostu ziarna na czas rozwoju ciasta

Similarly as in the earlier studies [Dojczew et al. 2004, 2005] bread baked from flour from pre-harvest sprouted grain showed poorer palatability and physico-chemical properties. The loaves were of insufficient volume, crust uneven, dark brown and thicker. In turn, the crumb showed an insufficient elasticity, uneven porosity and considerable viscosity. The bread obtained had an unusual, sweetish taste. These faults were

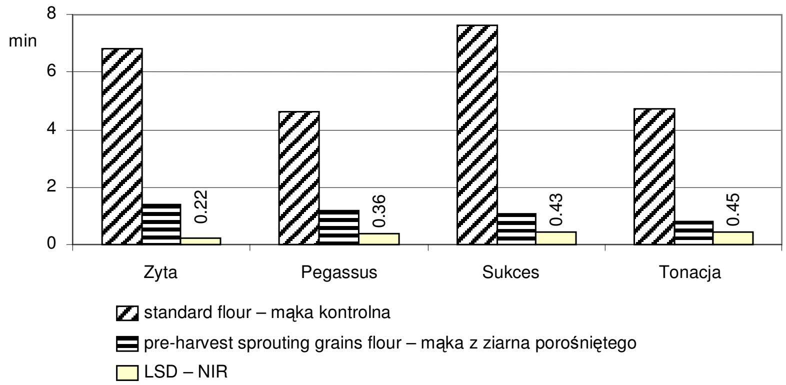

Fig. 4. The effect of the pre-harvest sprouting of grains on the stability of dough
Rys. 4. Wpływ porostu ziarna na czas stałości ciasta

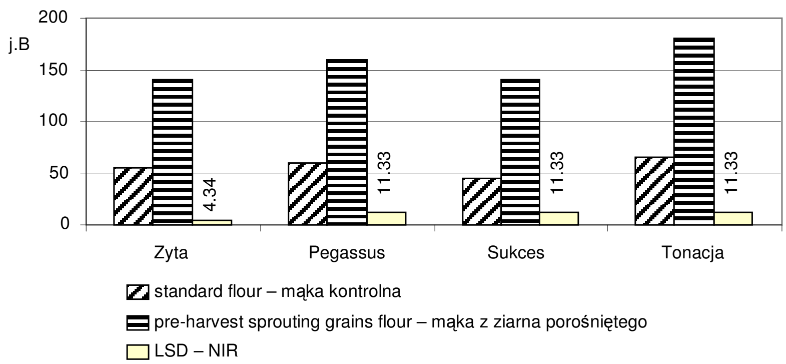

Fig. 5. The effect of the pre-harvest sprouting of grains on the softening of dough
Rys. 5. Wpływ porostu ziarna na rozmiękczenie ciasta

caused by the processes of saccharification and starch dextrinisation as well as a change in the quantity and quality of gluten proteins occurring during sprouting as result of an increased amylolytic and proteolytic activity.

The biochemical changes caused by sprouting resulted also in a statistically significant decrease of the volume of bread. In turn, the acidity of flour from sprouted grain increased significantly due to an increase in the content of fatty acids, phosphates and free amino acids caused by the activity of esterases and peptidases (Figs 6 and 7).

Of the improvers used vital gluten had a better effect, increasing the volume and porosity of bread obtained from flour from pre-harvest sprouted grain.

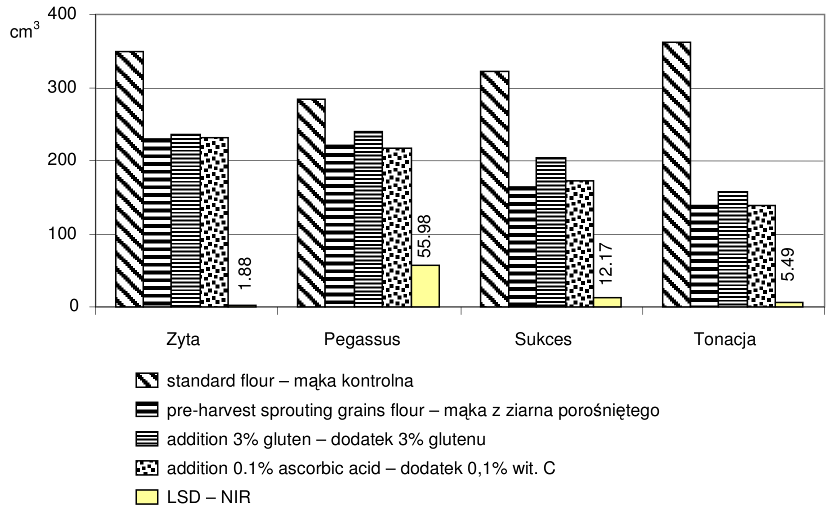

Fig. 6. The effect of the pre-harvest sprouting of grains on the volume of 100 g bread
Rys. 6. Wpływ porostu ziarna na objętość 100 g pieczywa

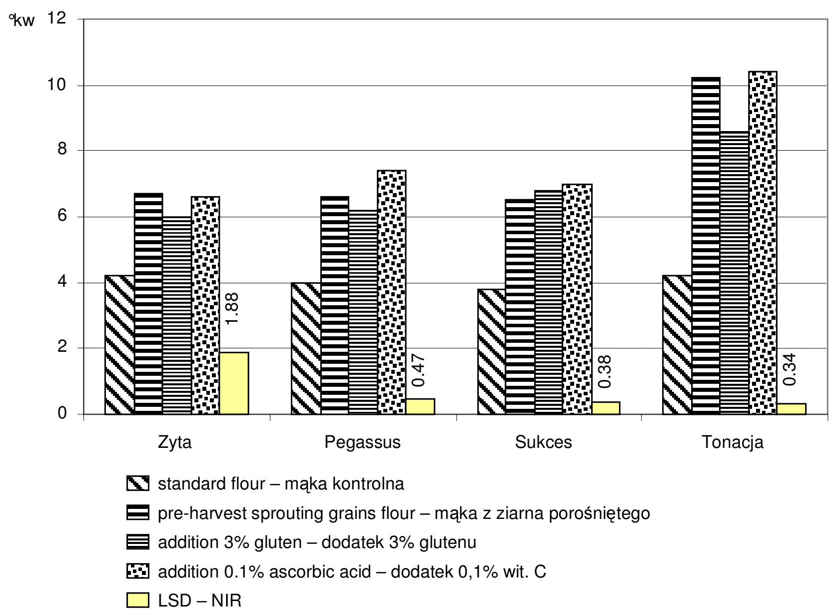

Fig. 7. The effect of the pre-harvest sprouting of grains on the acidity of crumb
Rys. 7. Wpływ porostu ziarna na kwasowość miękiszu

CONCLUSIONS

1. As result of pre-harvest sprouting of grain there occurred an overall increase in the proteolytic activity in all milling fractions of the varieties examined. The values of this indicator were observed to be higher in fine and bruise bran than in flour. Simultaneously, the content of non-protein nitrogen increased proportionally to the activity of proteases in each fraction examined.
2. The increased proteolytic activity in samples of pre-harvest sprouted grain resulted in a decrease of the quantity of gluten and its poorer physical properties, which was reflected by an increased spreadability.
3. The physico-chemical changes taking place in the flour from pre-harvest sprouted grain had an unfavourable effect on the rheologic properties of dough, as shown by a decrease in the water holding capacity of flour, shorter dough development and stability period and an increased softening in all the samples analysed.
4. Bread obtained from flour from pre-harvest sprouted grain showed two types of faults: physical, expressed by a considerable decrease in the volume of the loaf and palatability – the crust was dark brown and coarse, the crumb was characterised by an uneven porosity, considerable viscosity and sweetish taste. The use of improvers improved the quality of bread, but better effects were obtained when introducing vital gluten.

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WPŁYW AKTYWNOŚCI PROTEOLITYCZNEJ NA WARTOŚĆ TECHNOLOGICZNĄ MĄKI PSZENNEJ Z ZIARNA POROŚNIĘTEGO

Streszczenie. Badania dotyczyły poziomu ogólnej aktywności proteolitycznej we frakcjach mąki oraz otrąb drobnych i grubych, otrzymanych z czterech odmian pszenicy (‘Zyta’, ‘Pegassus’, ‘Sukces’, ‘Tonacja’), poddanych procesowi porastania. Prześledzono również wpływ procesu porastania na właściwości funkcjonalne mąki, określając cechy fizyczne glutenu i ciasta. Analizy obejmowały oznaczenie zawartości białka ogółem, azotu niebiałkowego, glutenu mokrego i aktywności proteolitycznej oraz określenie cech reologicznych ciasta. Badania zakończono próbnym wypiekiem laboratoryjnym z zastosowaniem witaminy C i glutenu witalnego jako polepszaczy do mąk z ziarna porośniętego. We wszystkich frakcjach przemiału wskutek porostu wzrosła ogólna aktywność proteolityczna, szczególnie dużą aktywnością odznaczała się odmiana ‘Tonacja’. Jednocześnie we wszystkich badanych frakcjach obserwowano wyższy poziom azotu niebiałkowego. Mąka otrzymana z ziarna porośniętego charakteryzowała się zwiększoną wodochłonnością, a ciasto pogorszeniem cech reologicznych. Pieczywo uzyskane z mąk z ziarna porośniętego było niedostatecznej jakości. Zastosowane do wypieku polepszacze (gluten witalny i witamina C) na ogół wpływały na korzystne zmiany organoleptyczne i fizykochemiczne w pieczywie.

Słowa kluczowe: pszenica, porost ziarna, aktywność proteolityczna, wartość wypiekowa

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