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THE CHANGES OF SOME ENZYMES ACTIVITIES DURING GERMINATION OF RYE KERNELS

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ABSTRACT

The effect of germination on the rate of formation of some endo- and exohydrolases, important in baking, in rye kernels was examined. Germination of the rye gave increase in α-amylase, endo-β–xylanase, endo-β-glucanase, β-xylosidase, α–arabinosidase, β-glucosidase as well as endo- and exopeptidases activities. The most intensive changes of activities in the group of examined enzymes were observed for α–amylase. β-amylase activity did not significantly increase on germination. The increase of endohydrolases activities in germinating rye kernels was much more markedly compared with activities of exohydrolases.

Key words: rye, germination, endoenzymes and exoenzymes

INTRODUCTION

In some years germination of rye before harvest is a major problem in central and northern Europe [26]. Pre-harvest sprouting damage of rye grain may cause considerable losses in its processing quality. Good quality ungerminated rye contains low concentrations of different hydrolytic enzymes, of which amylases, NSP-degrading enzymes and proteases are the most technologically important in baking [22]. The concentration of most these enzymes increases during germination of rye kernels, and their activity so alters the functional properties of starch, pentosans, β-glucans and proteins as to make rye flour unsuitable for bread production [22, 26]. Many investigations have been focused on enzymes in germinating wheat and barley [5, 14, 15, 17, 27]. In contrast rye enzymes produced in the early stages of germination have not been well characterised. Furthermore the most of the previous researches on germinating or sprouting rye has been attributed to the production and

synthesis of amylolytic enzymes, its inhibitors, increase their activities and changes in the amylase-starch system [6, 7, 8, 20, 25]. Also only very little is know about the NSP-degrading enzymes (pentosanases and glucanases) and proteolytic enzymes in germinated rye.

The purpose of this study was to examine the effect of rye germination under controlled conditions on the rate of formation of endo- and exohydrolases, some of which may play an important role in rye bread production.

MATERIALS AND METHODS

MATERIALS

One polish variety of winter rye Dankowskie Złote was selected for a study of the changes in enzyme activity of rye that occurs during kernel germination. Dankowskie Zlote is the predominant cultivar of rye in Poland. The sample of rye was obtained from Plant Breeding Station in Laski after the 1999 harvest.

METHODS

Analyses of sound (ungerminated) rye

The content of sprouted kernels in the rye sample, falling number and amylographic evaluation were determined according to standard methods: ICC No. 103/1, 107 and 126, respectively [9]. The maltose value was determined by the method of Rumsey and Ritter [23]. Total nitrogen was determined by the Kjeldhal method [23]. Protein content was obtained by multiplying the nitrogen content by 6.25.

Germination of rye

Rye kernels were surface sterilised by soaking in a solution of 2.0% (w/v) sodium hypochloric for 30 min at 20°C. The kernels were washed many times with sterilised water. They were soaked for 24 h in distilled water at 4°C with two water changes and germinated on wet filter paper in a germination cabinet at 20°C and at 100% humidity. At intervals of 12, 24, 36, 48, 72 and 96 h the kernels were withdrawn and frozen at -30^{o}C and then freeze-dried. Roots were removed. The freeze-dried kernels were ground to pass a 0.8 mm sieve using the laboratory mill (Falling Number 3100) and stored at 4^{o}C. Three parallel assays were performed for each time of germination.

Enzymes activities assays

α-Amylase assay. α-Amylase (EC 3.2.1.1.) activity was determined according to ICC Standard Method No. 108 [9].

β-*Amylase assay*. β-Amylase (EC 3.2.1.2.) activity was determined using p-nitrophenyl-α-D-maltopentaoside (PNPG5) as substrate by the method of McCleary and Codd [18]. One unit of β-amylase activity was defined as the amount of enzyme which releases 1 micromole of p-nitrophenol per minute under defined assay conditions.

Endo-(1→4)-β-*xylanase assay*. Endo-(1→4)-β-xylanase (EC 3.2.1.8.) activity was assayed by the method of Biely et al. [2] using 4-O-methyl-D-glucurono-D-xylan-Remazol brilliant blue R (RBB) as substrate. One unit of activity endo-β-xylanase was arbitrarily defined as a change in absorbance of 0.1 at 595 nm per 60 min per g wholemeal rye flour.

Glycosidases assay. β-D-Glucopyranosidase (EC 3.2.1.21), β-D-xylopyranosidase (EC 3.2.1.37) and α-L-arabinofuranosidase (EC 3.2.1.55) activities were assayed using p-nitrophenyl-β-D-glucopyranoside, p-nitrophenyl-β-D-xylopyranoside and p-nitrophenyl-α-L-arabinofuranoside as substrates, respectively as described by Corder and Henry [5]. One unit of glycosidase activity was defined as the amount of enzyme which releases 1 micromole of p-nitrophenol per minute per g wholemeal rye flour.

Endo-(1→3),(1→4)-β-*glucanase assay*. Endo-(1→3),(1→4)-β-glucanase (EC 3.2.1.73) activity was determined using azo-barley β-glucan as substrate and according to assay procedure Megazyme Ltd. [19]. One unit of endo-β-glucanase activity was defined as micromole of glucose reducing sugar equivalent liberated per minute per g wholemeal rye flour under defined assay conditions.

Endoproteolytic assay (azocasein hydrolyzing activity). Endopeptidase activity was determined using the modified azocasein assay of Kruger [11]. One unit of endopeptidase activity was defined as a change in absorbance of 0.01 at 440 nm after 120 min under standardised assay conditions.

Exoproteolytic assay (hemoglobin hydrolyzing activity). Exopeptidase activity was determined by the Bushuk et al. [4] modification of the Ayre-Anderson method. Soluble nitrogen products were assayed by the procedure of Lowry et al. [13], using tyrosine to obtain the standard curve. Exopeptidase activity was expressed in micromoles of tyrosine per minute per g wholemeal rye flour.

All presented results are the mean values of at least three replications. The mean results and the standard deviations of mean values are given in table and figures.

RESULTS AND DISCUSSION

The basic quality characteristics of rye cv. Dankowskie Zlote are shown in Table 1. The rye sample did not contain of sprouted kernels. It was classified as "bread rye" with moderate amylolytic activity and with good breadmaking potential properties [22].

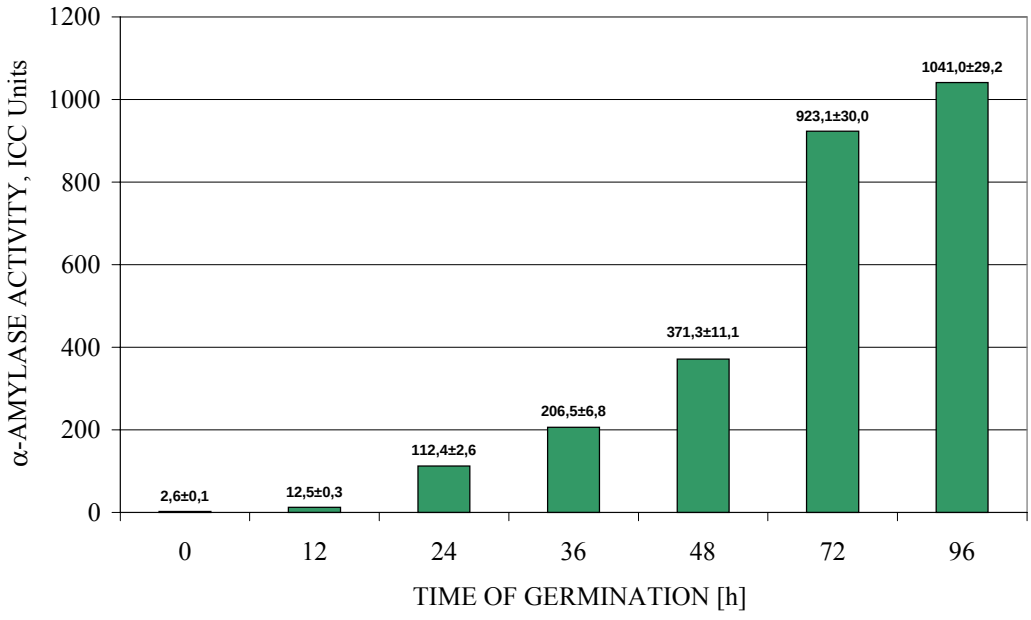
Table 1. Quality characteristics of ungerminated rye

Quality characteristics	Rye cv. Dankowskie Zlote
Sprouted kernels (%)	0
α - Amylase activity (ICC Units)	2.6±0.1
Falling number (s)	168±7
Amylographic evaluation:	
- Peak viscosity (BU)	680±25
- Peak temperature (°C)	67±1
Maltose value (% d.b.)	2.5±0.1
Protein (N x 6.25) (% d.b.)	10.4±0.2

AMYLASES

The detrimental effect of post-maturity germination of rye is attributed first of all to changes in the amylase-starch system. Changes that occurred in the α-amylase activity of Dankowskie Złote rye during kernels germination is shown in Figure 1.

Figure 1. Changes in α-amylase activity during germination of rye



The most intensive changes of activities in the group of all examined hydrolytic enzymes were observed for α-amylase. Activity of this endoamylase increased significantly over the first three days but showed a smaller increase on the forth day of germination. The level of α-amylase in rye increased 355- and 400-fold after 72 and 96 hours of germination, respectively. Only a minor rise in activity was detected for β-amylase. Activity of this exoamylase increased from 2813±67 to 2991±62 U on germination for 96 hours. In general, these results agree with few reports on the increase in amylolytic activity of rye kernels during germination [20, 21, 25]. It is well know that α-amylase is generally recognised as one of the most important germinative enzymes of rye. Upon germination of rye kernels, the level of α-amylase activity can increase several hundred-fold depending on cultivar, location and assay method [12, 21, 22, 25]. Lee and Unrau [12] reported that the activity of α-amylase increase logarithmically with time of rye germination. The presented results have not confirmed this relationship. The smaller increase in activity of β-amylase on germination is likely to be due to the fact that ungerminated rye contains an abundance of β-amylase. Furthermore a large part of β-amylase of ungerminated cereals is known to occur in a latent and insoluble form. During germination of cereal kernels, the amount of free β-amylase increases while the amount of latent enzyme decreases [10]. From a technological point of view rye with very low as well as high amylases activities is not acceptable for production of good quality rye bread. Rye with low and moderate amylases activities is most suitable for breadmaking [22].

NON-STARCH POLYSACCHARIDES-DEGRADING ENZYMES

The major non-starch polysaccharide (NSP) constituents of the aleurone and endosperm cell-walls of rye are pentosans (mainly arabinoxylans) and mixed-linkage(1→3),(1→4)-β-glucan. It has been reported that three groups of enzymes are important in the degradation of arabinoxylan: endo-(1→4)-β-xylanase, β-D-xylopyranosidase and α-L-arabinofuranosidase and two enzymes in the decomposition of (1→3),(1→4)-β-glucan, including endo-(1→3),(1→4)-β -glucanase and β-D-glucopyranosidase [7, 8]. In this study all of these enzymes have been detected in ungerminated as well as in germinated rye. Comparisons of the both endo-polysaccharases (endo-(1→4)-β-xylanase and endo-(1→ 3),(1→4)-β-glucanase) activities during germination of rye kernels are presented in Figures 2 and 3.

Figure 2. Changes in endo-(1→4)-β-xylanase activity during germination of rye

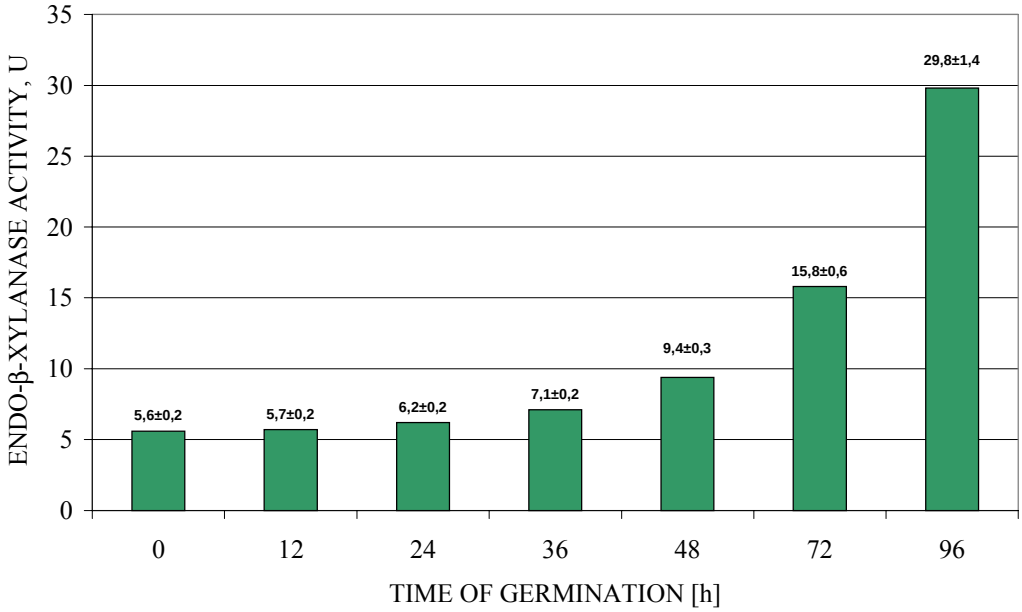
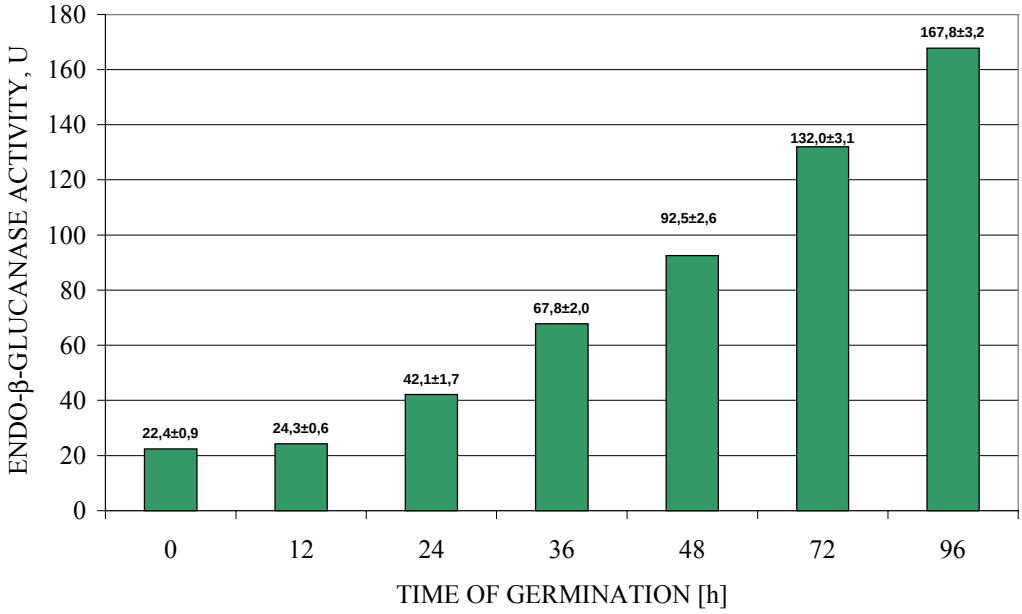
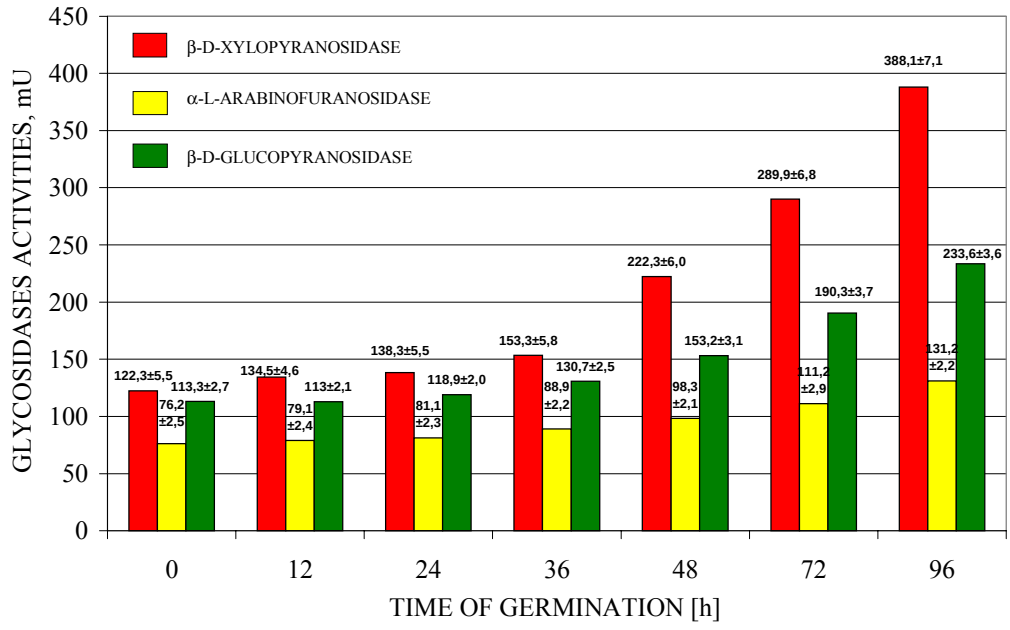


Figure 3. Changes in endo-(1→3),(1→4)-β-glucanase activity during germination of rye



The activity of endo-(1→3),(1→4)-β-glucanase increased earlier and at a faster rate than the activity of endo-(1→4)-β-xylanase. The activity of endo-(1→3),(1→4)-β-glucanase in rye kernels increased throughout the 96 h of germination whereas the level of endo-(1→4)-β-xylanase was relatively low over the first 48 h but increased quickly after 72 h. Total increase of activities of these enzymes after 96 h of germination was 7.5-fold and 5.3-fold, respectively. There is no evidence that endo-(1→3),(1→4)-β-glucanase is produced before α-amylase as it has been reported for barley [15]. The β-glucanase activities of various germinating cereals have been compared by Stuart et al. [24]. The highest activity was observed in germinating barley, but significant activity was also found in germinating kernels of rye. Endoxylanase activity in germinated rye kernels has also been studied by Karlsson [8] and by Namjiljav [20]. It has been found a marked rise in activity of endoxylanase measured viscometrically after three days of rye germination using soluble pentosan as a substrate [8]. The activities of the three exo-polysaccharases (β-D-xylopyranosidase, α-L-arabinofuranosidase and β-D-glucopyranosidase) against of nitrophenyl glycosides during germination of rye are shown in Figure 4.

Figure 4. Changes in glycosidases activities during germination of rye



During germination the activities of all three glycosidases increased at different rates. The activity of β-D-xylopyranosidase increased markedly after 36 h of germination however the activities of both α-L-arabinofuranosidase and β-D-glucopyranosidase increased gradually during 96 h. After 96 h the level of β-D-xylopyranosidase found in this study was significantly higher than the levels of both α-L-arabinofuranosidase and β-D-glucopyranosidase (3.2-, 2.1- and 1.7-fold respectively). These increases in activities of glycosidases after germination of rye are much larger compared with those observed in germinating wheat kernels reported by other workers. Namjiljav [20] showed that a rapid 20-fold rise of β-D-xylopyranosidase in rye, and an 6-fold rise of α-L-arabinofuranosidase in wheat on the fifth day of germination, whereas Marsh et. al. [17] found no increase in α-L-arabinofuranosidase activity, and only slight increase in β-D-xylopyranosidase activity as well as significant rise in β-D-glucopyranosidase activity after germination of wheat. The β-xylopyranosidase, α-amylase and protease activities of sprout-damaged rye have been compared by Fretzdorff [6]. She observed higher β-xylopyranosidase activity in sprouted rye with higher α-amylase and protease activities. From a technological point of view, it is interesting to note that the high activities of NSP-degrading enzymes might facilitate the movement of other hydrolytic enzymes from the periphery layers of kernel to the centre of the rye endosperm. Moreover enzymatic damaged NSP such as pentosan and β-glucan affecting the baking quality of rye flour adversely [22].

PROTEASES

Changes in both endo- and exoproteolytic activities, using azocasein and hemoglobin as substrates, during the germination of rye kernels are shown in Figures 5 and 6.

Figure 5. Changes in endoproteolytic activity during germination of rye

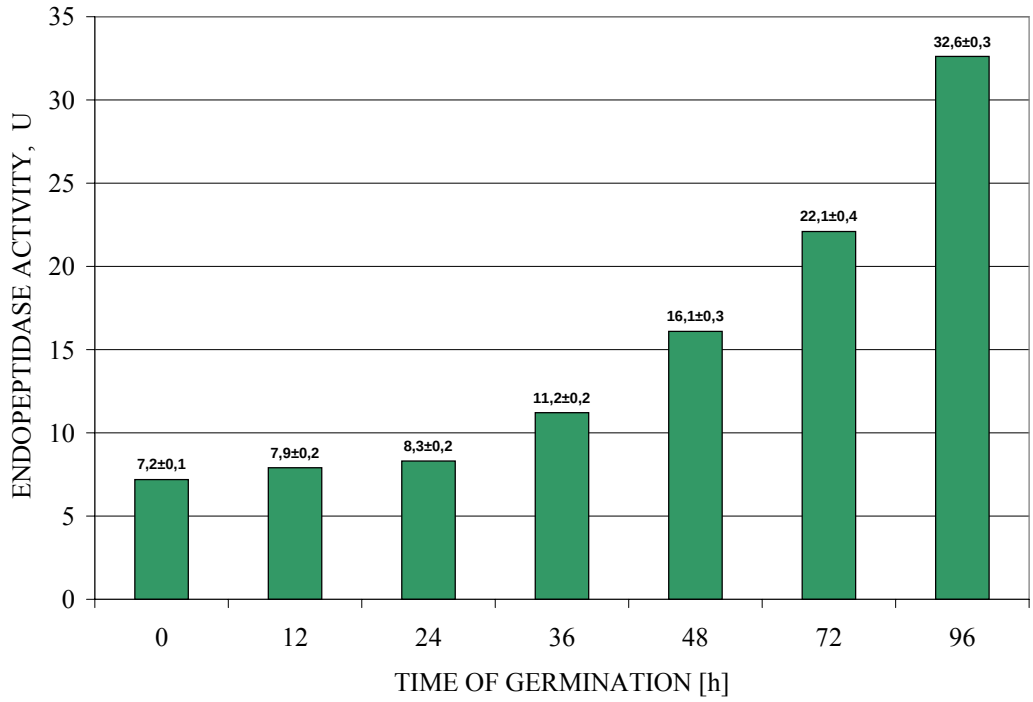
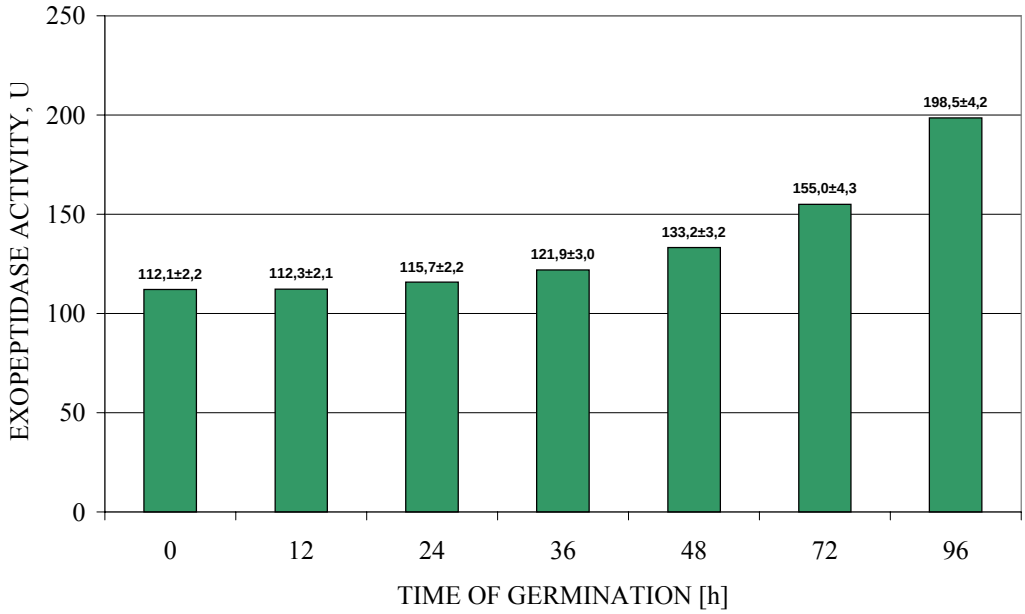


Figure 6. Changes in exoproteolytic activity during germination of rye



The results obtained revealed the rise in activities of both proteolytic enzymes with germination of rye. However the increase in exoproteolytic activity was only small (1.8-fold increase in exoproteolytic activity after 96 hours of germination). Larger increase was found for endoproteolytic activity during germination using azocasein as substrate. There was a relatively little change in activity of endopeptidase over the first 36 h of germination but it showed rapid increases at later stages (after 36 h) of the process. Increase in endopeptidase activity occurred earlier and was more pronounced than in activity of exopeptidase. The total endoproteolytic activity raised 4.5-fold after 96 h of germination. Madl and Tsen [16] showed that the exoproteolytic activity of rye flour obtained from ungerminated grain is greater than that of wheat flour, and close to that of triticale flour. The results presented here are generally consistent with previous studies [3, 20]. Brijs et al. [3] showed that the proteolytic activity in rye after three days of germination was about 5.0-fold higher than that in ungerminated rye.

CONCLUSIONS

The results showed that ungerminated and germinated rye contain relatively broad spectrum of hydrolytic enzymes. The α-amylase is a predominant enzyme in germinated rye kernels, whereas the β-amylase activity is practically stable during germination under controlled conditions. The NSP-degrading enzymes are not formed at earlier stages of germination than the α-amylase. Among the NSP-degrading enzymes the activity of endo-(1→3),(1→4)-β-glucanase increases earlier and at faster rate than the activity of endo-(1→4)-β-xylanase. Furthermore the level of β-D-xylopyranosidase is significantly higher than the levels of both α-L-arabinofuranosidase and β-D-glucopyranosidase. Endopeptidase activity increases more than exopeptidase activity. Finally, it is interesting to note that the increase in all examined endohydrolase activities during germination of rye kernels is much more pronounced than the rise in exohydrolase activities. From a practical point of view this might be the main reason that flour recoverable from sprouted rye is practically unacceptable for bread production.

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