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CHANGES IN α-AMYLASES ACTIVITY IN WHEAT AND MALTED WHEAT GRAIN AFTER LONG STORAGE

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Key words: wheat, stored wheat, malted wheat grain, amylolytic activity.

The effect of prolonged storage on α-amylases activity in wheat and malted wheat grain was studied. It was demonstrated that four-year storage increases the activity of α-amylases and at the same time decreases the total activity of combined α- and β-amylases and the protein level.

INTRODUCTION

Alpha-amylases, i.e. endoamylases — C.3.2.1.1. (4 glucan-hydroxylases α-1,4 glucan), constitute a sizeable fraction of biologically active proteins in cereal caryopses. They are noteworthy in view of their role in starch hydrolysis and their effect on rheological and technological properties of flour [5].

The available literature contains no univocal data that would explain the effect of storing the grain on the activity of its endogenous α-amylases. This is mostly due to the fact that the majority of amylolytic activity measurement methods do not enable the specific determination of this activity. Such a situation prompted the present study of the effect of long-term storage on the activity of α-amylases in wheat and malted wheat grain.

MATERIAL AND METHODS

MATERIAL

The study was performed with grain of the Aria variety of winter wheat harvested on experimental fields of the Research Centre for Testing Varieties in Słupia Wielka (Poznań voivodship, Poland). Malted grain

was obtained according to [5] with 96-h germination for four-day malt and 144-h germination for six-day malt. The wheat and malted wheat grain was stored in tightly closed containers in controlled conditions (average temperature 20°C and 74% relative humidity) for four years. The basic physicochemical composition of Aria wheat grain and malted grain is given in Table 1.

Table 1. The effect of long storage on basic physicochemical composition of wheat and malted wheat grains and on the falling number

Raw material	Time of storage (years)	Moisture content %	Protein content (N × 5.7) in % d.w	Falling number S
Wheat grain	1976	13.7	12.50	360
	1980	13.8	12.00	307
Malted wheat grain without GA_3	1976	6.0	12.00	—
	1980	7.9	11.80	—
Malted wheat grain with GA_3	1976	6.3	12.00	—
	1980	7.6	11.77	—

The previously studied grain and malts obtained therewith [9] were of good quality and acceptable microbiologically.

METHODS

Moisture in the grain and malts was determined according to the Polish Standard PN 65 R-74006. Protein content was determined by the Kjeldahl method on a Tecator (Sweden) apparatus using the coefficient of conversion to protein K = 5.7. The falling number in grain was determined by the 56-81 B method according to [1]. Protein content in the extracts was assayed by the method of Lowry et al. [4]. Standard curves were plotted for egg albumin and lysozyme. Similar results for both proteins were obtained at light wavelength of 750 nm. Total activity of combined α- and β-amylases was measured according to the method of Bernfeld [2] with modifications described by Warchalewski and Tkachuk [10]. The activity of α-amylase was measured as described earlier in [10].

EXTRACT PREPARATION

50-g (dry substance) portions of wheat caryopses and malts were ground in an ML 155 laboratory grinder and then shaken on a type 327 vibration shaker in a twice higher volume of 0.001 M calcium acetate. Extraction was performed at 20°C for four hours in media with pH 6.3-6.5

(wheat), or 5.8 (malts containing gibberelic acid, GA_3) or 6.1 (malts without GA_3). The suspensions were then centrifuged for 15 min at 6000×G and temperature 4°C in a type K-24 centrifuge, and the precipitate was discarded. In the supernatant, termed the "crude extract", we determined the protein content and the total activity of combined α- and β-amylases. The heated extract was obtained from the respective crude extracts, adjusting pH to 7.0 with 4% ammonium hydroxide and heating for 10 min at 70°C and then rapidly cooling in an ice water bath. The precipitated sediment was centrifuged at 9000×G for 10 min at 4°C and discarded. In this supernatant, called the "heated extract, we assayed protein concentration and α-amylases activity. It was demonstrated earlier [10] that in an extract thus prepared the activity of β-amylases is stopped completely, while at least 80% of the activity of endogenous α-amylases is retained.

Protein content, total activity of combined α- and β-amylases, and α-amylase activity were analysed in extracts of wheat caryopses and in the obtained two kinds of malts (the four-day with an addition of 0.1 mg GA_3 per kg, and the six-day without GA_3) using extinction read-outs from a Spekol photocolorimeter equipped with an EK-1 attachment. In view of the after-ripening effect, all analyses were performed three months after the wheat harvest, assuming that after this time all biochemical changes in grain components will cease; the analyses were repeated after four years of grain storage.

RESULTS AND DISCUSSION

Table 1 shows the effect of prolonged storage on the basic physicochemical composition of wheat and malted wheat grain and on the falling number.

A 4% drop of total protein content was noted in the stored wheat grain together with a much smaller, 2% on the average, decrease of this content in both stored malts. Much higher protein losses were observed in the case of protein extracted with calcium acetate (Table 2).

The results of biochemical analyses of the obtained wheat and malted wheat grain extracts before and after storage are given in Table 2.

The amount of protein determined in both the crude and the heated extracts was markedly lower after four years of sample storage. It was also observed that in the case of wheat and its malt with GA_3, the drop in the level of protein soluble in calcium acetate was very similar, amounting to 15 and 17%, respectively in crude extracts, and 13% in heated extracts. A much higher protein loss occurred in the malt without GA_3, namely 28% in the crude axtract and 23% in the heated extract. Bolling et al. [3] also found a drop in the content of protein soluble in sodium acetate in stored wheat, with the total protein content in grain remaining

Table 2. Changes in contents of protein, combined α and β-amylase activity and α-amylase activity in wheat and malted wheat grain during long storage (100 g grain/dry weight)

Raw material	Time of storage (years)	Crude extracts			Heat-treated extracts		
		protein (mg)	total activity of combined α and β-amylase (UAA)	specific activity of combined α and β-amylase (UAA) mg protein	protein (mg)	total activity of α-amylase (UAA)	specific activity of α-amylase (UAA) mg protein
Wheat grain	1976	1375.0	35 842	26.07	973.8	266	0.27
	1980	1175.0	26 434	22.50	850.0	448	0.53
Malted wheat grain without GA_3	1976	2450.0	78 136	31.89	1836.7	18 363	10.00
	1980	1752.0	50 753	28.97	1416.0	13 118	9.26
Malted wheat grain with GA_3	1976	2325.0	73 118	31.45	1662.5	14 471	8.70
	1980	1920.0	53 333	27.78	1440.0	12 043	8.36

unaltered. It was demonstrated at the same time that there is a dependence between the decrease of the content of protein determined in stored samples of wheat and malt and the drop in total activity of combined α- and β-amylases in these samples, and also the drop in α-amylases activity in malt samples only (Table 2). After four years of sample storage the total activity of combined α- and β-amylases in wheat grain and malt with GA_3 decreased similarly, by 26% and 27%, respectively, while in the malt without GA_3 by as much as ca. 35%. On the other hand, the activity of α-amylases in malts with and without GA_3 decreased to a lesser extent, by 17% and 29%, respectively. The close dependence between the drop of protein content and the discussed amylolytic activity is due to the fact that the studied proteins soluble in calcium acetate contain mostly amylolytic enzymes.

In earlier studies, Warchalewski and Tkachuk [10] demonstrated electrophoretically that the proteins extracted from malted wheat grain with calcium acetate consisted mainly of isoenzymatic forms of α-amylases. Hence, the changes occurring in these proteins must have been followed by changes in their activity.

Particularly noteworthy is the established very substantial increase (by 68%) of the activity of α-amylases in the heated extract of stored wheat grain, accompanied by a simultaneous drop of protein content in this extract by ca. 13%. This 13% reduction of protein content can probably be related, in part at least, with changes modifying the α-amylases-inhibitors complex which can lead to an activation of a part of the till

now inactive α-amylases, as it was earlier suggested by Warchalewski [5]. The observed marked increase of α-amylase activity in stored wheat supports an earlier conception about the occurrence of a part of the endogenous α-amylases in wheat grain in the form of inactive complexes with their inhibitors [5]. This theory is also confirmed by studies demonstrating a considerable increase of α-amylases activity in wheat depending on extraction conditions [7], studies of soaked wheat [6], and analyses of the dynamics of changes of α-amylases and inhibitor activities during controlled malting [8].

CONCLUSIONS

1. The amount of protein soluble in calcium acetate, both in crude and in heated extracts of Aria wheat, decreased after four years of grain storage.

2. The drop of protein content in stored wheat and malted wheat grain samples depended on the drop of total activity of combined α- and β-amylases, but only in the malt samples.

3. A marked increase of α-amylases activity (by 68%) together with a simultaneous drop in the amount of extracted protein (by about 13%) was observed in the stored grain.

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LITERATURE

1. American Association of Cereal Chemists. Approved Methods, AACC, St. Paul Mn, 1976, 2.
2. Bernfeld O.: Methods in Enzymology, S.O. Colwich N.O.: Kaplan Academic Press, New York 1955, 1, 149.
3. Bolling H., Weipert D., El. Baya A. W.: Landwirtsch. Forsch Kongressband 1977, part I, 182.
4. Lowry O. H., Rosenbrough N. Y., Farr A. L., Randall R.: J. Biol. Chem., 1951, 193, 265.
5. Warchalewski J. R., Roczniki AR-Poznań 1978, 82, 1.
6. Warchalewski J. R., Baranowska U., Madaj D.: XII Sesja Naukowa Kom. Techn. i Chemii Żywności PAN Lublin, 11-12 II 1981, 64.
7. Warchalewski J. R., Madaj D., Jarosz Ł.: XVII Zjazd Polsk. Tow. Bioch., Warszawa, 11-13 IX 1980, 309.
8. Warchalewski J. R., Madaj D., Stasińska B., Skupin J.: XVI Zjazd Polsk. Tow. Biochem., Łódź, 7-9 IX 1980, 44.
9. Warchalewski J. R., Madaj D., Stasińska B., Skupin J.: Report of the realization of ministerial project MNSzWiT No. II-8, Stage I, 1978, 1.
10. Warchalewski J. R., Tkachuk R., Cereal Chem., 1978, 55 (2), 146.

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OCENA ZMIAN AKTYWNOŚCI α-AMYLAZ W PSZENICY I JEJ SŁODACH PO DŁUGOTRWAŁYM OKRESIE PRZECHOWYWANIA

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Streszczenie

Badano wpływ długotrwałego czasu przechowywania na aktywność amylolityczną w ziarnie pszenicy i jej słodach. Do badań użyto ziarno pszenicy ozimej odmiany ARIA oraz jej słody: 4-dniowy z dodatkiem kwasu giberelinowego (GA_3) i 6-dniowy bez GA_3. W ekstraktach z ziarniaków pszenicy i jej słodów oznaczono zawartość białka, łączną aktywność α i β-amylaz oraz aktywność α-amylaz, a także liczbę opadania. Wszystkie analizy wykonano 3 miesiące po zbiorze pszenicy i po 4 latach przechowywania ziarna. Stwierdzono, że 4-letni okres przechowywania ziarna w kontrolowanych warunkach wpływa na wzrost aktywności α-amylaz, jednocześnie obniżając łączną aktywność α i β-amylaz oraz poziom białka.