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BETA-GLUCAN ENZYMATIC HYDROLYSIS IN CEREAL GRAINS AND CEREAL PRODUCTS

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ABSTRACT

The effect of various conditions of enzymatic hydrolysis as well as selected glucanolytic and amylolytic enzymes on carbohydrate system changes, necessary for quantitative determination of beta-glucan was evaluated. Hydrolysis of beta-glucan should be followed by elimination of starch in the sample using alpha-amylase Termamyl 120L and alpha-amyloglucosidase enzymes.

It was found, that for beta-glucanase optimal hydrolysis time is 180 min, and concentration 2U/ml, while for beta-glucosidase 60 min and 6U/ml, respectively. The method is comparable to the standard recommended by ICC but less time consuming and free of errors caused by application of non-specific enzymes, however gives about 15% lower results to ICC method.

Key words: beta-glucan determination, dietary fiber, alpha-amylase, alpha-glucosidase, beta-glucanase, beta-glucosidase, enzymes, cereal grain, cereal products.

INTRODUCTION

Cereals constitute a valuable source of dietary fiber in everyday diet of a human being. Pentosans and beta-glucan are among the most important components of dietary fiber found in cereal and its products, especially in barley, oats and rye [2,5,6,8]. Not only the relation between beta-glucan consumption level and metabolic civilization diseases were found (e.g. arteriosclerosis, diabetes, large intestine cancer, obesity) but it was also proved that beta-glucan might be useful in treatment and prevention of such diseases [4,5,8]. This most substantial role played by beta-glucan in nutrition of a healthy human calls for precise determination of non-starch polysaccharides (NSP) fraction in cereal material.

The aim of this work is to scrutinize the changes taking place in a sample carbohydrate system during varying conditions of enzymatic hydrolysis as well as to assess the usefulness of selected glucanolytic and amylolytic enzymes for quantitative determination of beta-glucan content in cereal and cereal products.

MATERIALS AND METHODS

The following samples of grain were used to carry out the tests: barley of Nagrad variety, oats of Ułan variety, wheat of Henika variety, rye of Dańkowskie Złote variety, triticale of Bogo variety, commercial barley flakes and oats bran.

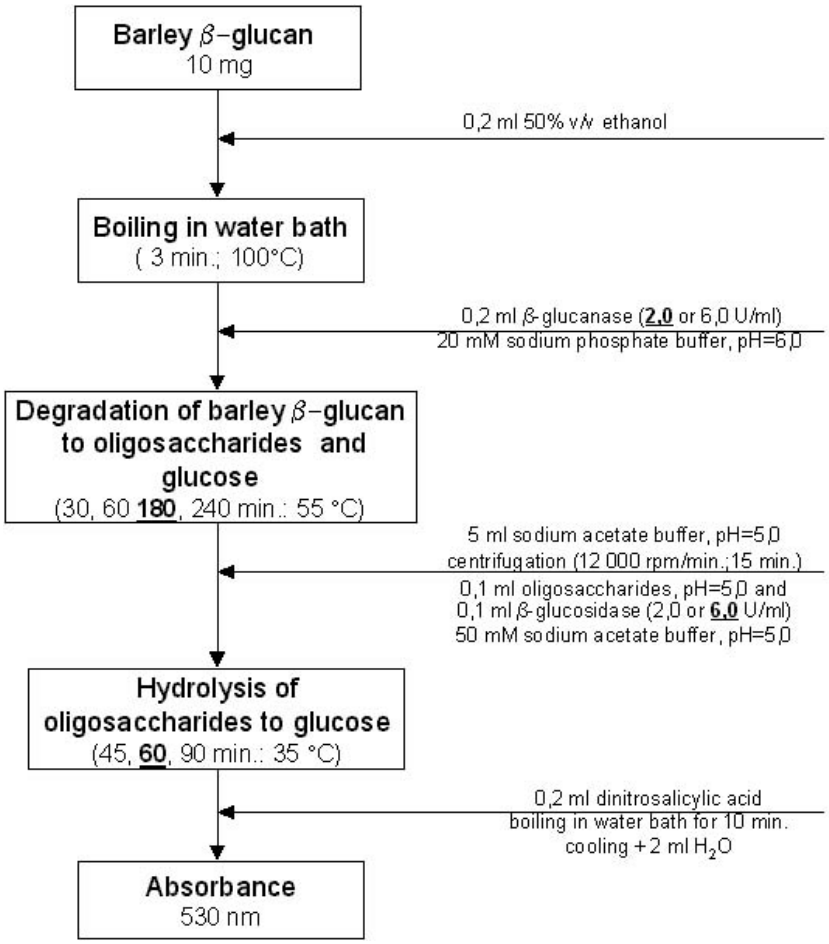
Barley and oats grain were dehulled manually. Barley and oats grain contained 10% and 25% of husk, respectively. Grain samples, barley flakes and oats bran were ground (<250 μm) and stored in hermetic boxes in temperature of 4°C.

Reagents: beta-glucanase from *Bacillus subtilis* (EC 3.2.1.6) (Fluka), beta-glucosidase from almonds (EC 3.2.1.21) (Fluka), thermostable alpha-amylase (EC 3.2.1.1) Termamyl 120 L (Novo A/S), alpha-amyloglucosidase from *Aspergillus* (EC 3.2.1.3) (BDH Biochemicals), beta-glucan from barley (Sigma), (1-3)(1-4) Beta-D-Glucan Assay Kit (Megazyme).
Other chemical reagents used in this experiment were analytical grade of purity.

For the purpose of beta-glucane determination by the method described herein it was necessary to adapt Hudson and Englyst's method [10] with 2-hydroksy 3,5-dinitrosalicylic acid (DNS) so that content of glucose released after beta-glucan enzymatic hydrolysis could be determined.

Model experiments. In the initial phase of the experiment the effect of concentration and digestion time of beta-glucanase (EC 3.2.1.6) and beta-glucosidase (EC 3.2.1.21) for the digestion rate of commercial beta-glucan preparation were tested. Figure 1 presents the conditions in which the experiment took place.

Figure 1. Model experiments flow chart



Furthermore, model experiments aimed at determining the influence of joint action of beta-glucanase and beta-glucosidase on hydrolysis degree of cereal starch. At this stage the influence of a two-step digestion of alpha-amylase (EC 3.2.1.1) - Termamyl 120 L and alpha-amyloglucosidase (EC 3.2.1.3) on hydrolysis degree of commercial barley beta-glucan preparation were also tested.

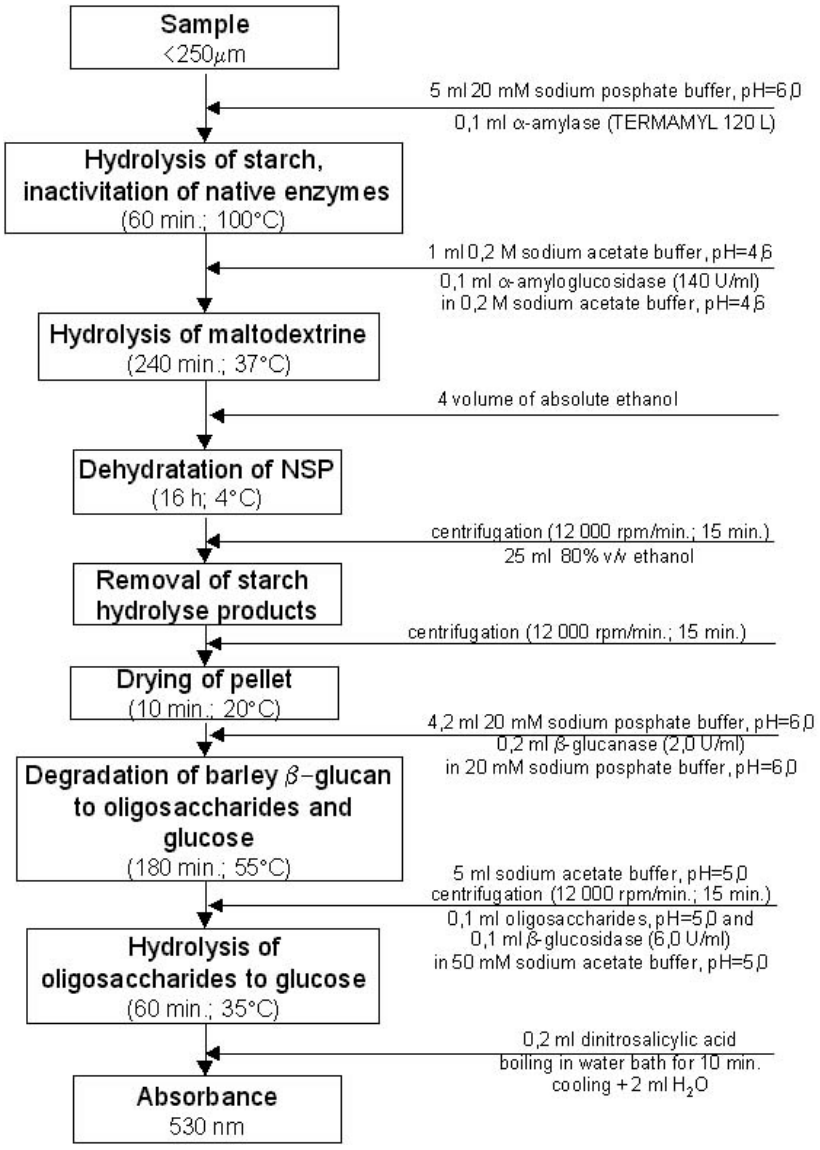
Parameters of beta-glucan enzymatic hydrolysis in cereal.

Usefulness of the method was initially tested on whole barley grain of Nagrad variety. Digestion time and concentration of beta-glucanase and beta-glucosidase on beta-glucan hydrolysis degree were tested. Different beta-glucanase concentrations, i.e. 0.5; 1.0; 2.0 and 6.0 U/ml as well as different samples hydrolysis time - 1, 2, 3, 4, 16 hours - were used. The same procedure was applied to determine the effect of beta-glucosidase on beta-glucan hydrolysis degree (enzyme concentration: 2.0 and 6.0 U/ml, incubation times: 45, 60 and 90 min).

In order to determine the content of glucose and glucooligosaccharides in a sample after its enzymatic digestion HPLC was used. The parameters of HPLC analysis were as follow: separation conditions - sample 100 µl (after filtration through 0.22 µm Millipore filter), column - Aminex HPX-87H (300 × 7,8 mm), eluent: 0.005 M H_2SO_4 0.6 ml/min, temperature of separation 45°C.

Figure 2 presents the results of preliminary experiments with optimal parameters of enzymatic hydrolysis and a flow chart for determination of beta-glucan content in cereal materials with different content of these polysaccharides.

Figure 2. Optimal parameters for determination of beta-glucan content in cereal and cereal products – a flow chart



RESULTS AND DISCUSSION

The aim of the model experiments was to determine optimal concentrations of beta-glucanase and beta-glucosidase, time the enzymatic hydrolysis of commercial preparation of barley beta-glucan and finally to check if the application of glucolytic enzymes is not accompanied by defragmentation of starch. In order to examine the degree of beta-glucan hydrolysis three different beta-glucanase and beta-glucosidase concentrations were used: (2 U/ml and 2 U/ml, 2 U/ml and 6 U/ml, 6 U/ml and 6 U/ml, respectively). Time of hydrolysis varied: 30, 60, 120, 180 and 240 min for beta-glucanase and 45, 60 and 90 min for beta-glucosidase (Figs. 3 and 4).

Figure 3. The effect of beta-glucanase (2U/ml) and beta-glucosidase (2U/ml) digestion time on hydrolysis degree of commercial beta-glucan preparation

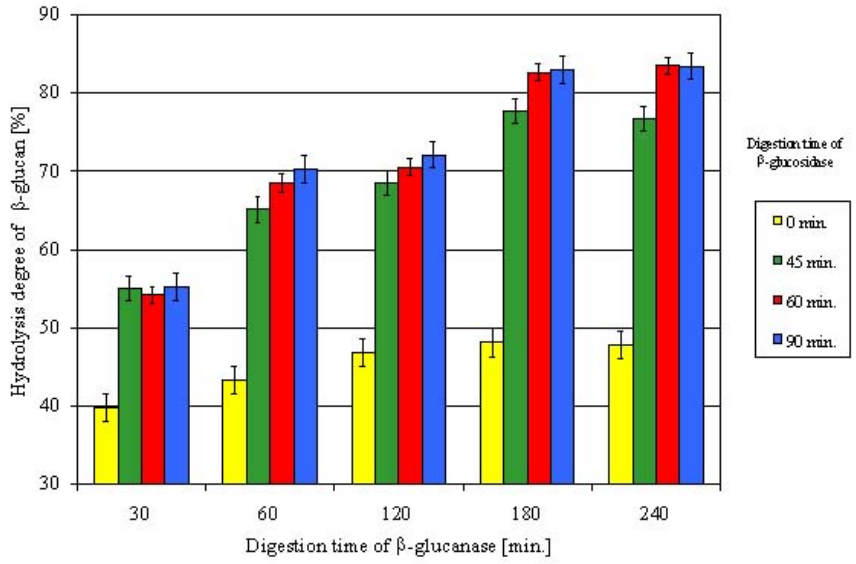
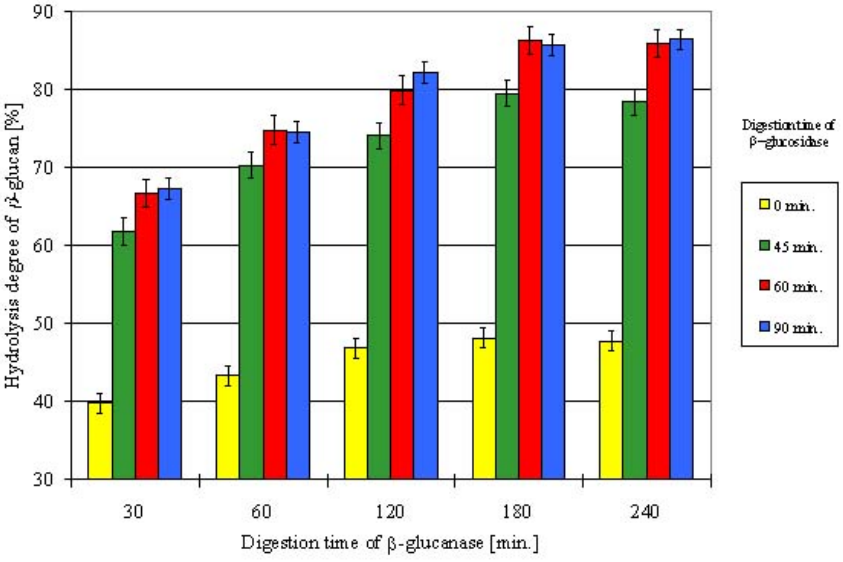


Figure 4. The effect of beta-glucanase (2U/ml) and beta-glucosidase (6U/ml) digestion time on hydrolysis degree of commercial beta-glucan preparation



The highest degree (86%) of beta-glucan break-down in the experiment conditions was achieved after a two-step hydrolysis, with beta-glucanase and beta-glucosidase. The concentration and hydrolysis time were 2 U/ml and 180 min, and 6 U/ml and 60 minutes for beta-glucanase and beta-glucosidase, respectively. One-step hydrolysis resulted only in a 50% of beta-glucan breakdown. Further prolongation of hydrolysis time or increasing of enzyme concentration did not lead to an increased degree of beta-glucan hydrolysis. Hydrolysis degree of commercial beta-glucan preparations originating from other manufacturers ranges from 70-98% [1].

The aforementioned parameters of enzymatic hydrolysis of beta-glucan preparation were used to determine the content of these polysaccharides in whole barley grain. Similarly to the model experiments, it was stated that neither increased enzyme concentration not prolonged hydrolysis time in relation to the previously set optimal values, substantially influences the content of beta-glucan (Figs. 5 and 6).

Figure 5. The effect of beta-glucanase (2U/ml) and beta-glucosidase (6U/ml) and digestion time on beta-glucan content in whole barley grain of Nagrad variety

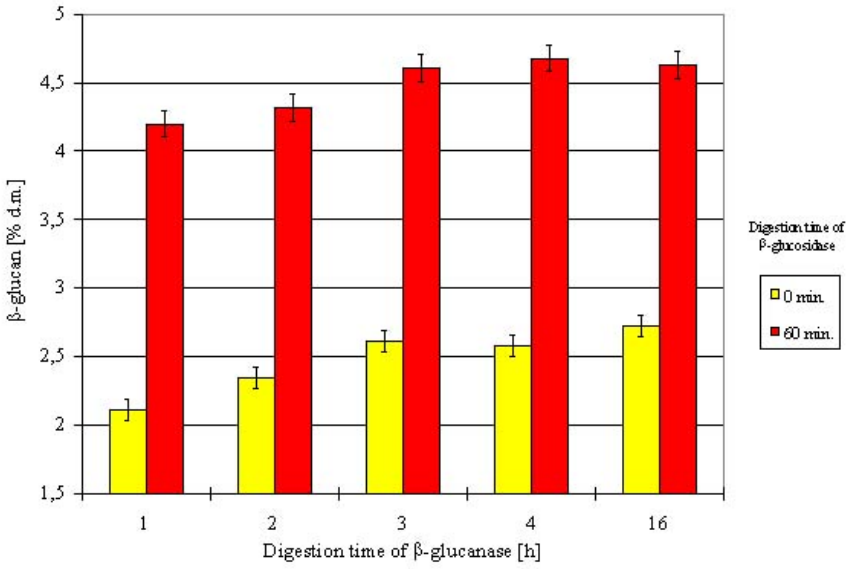
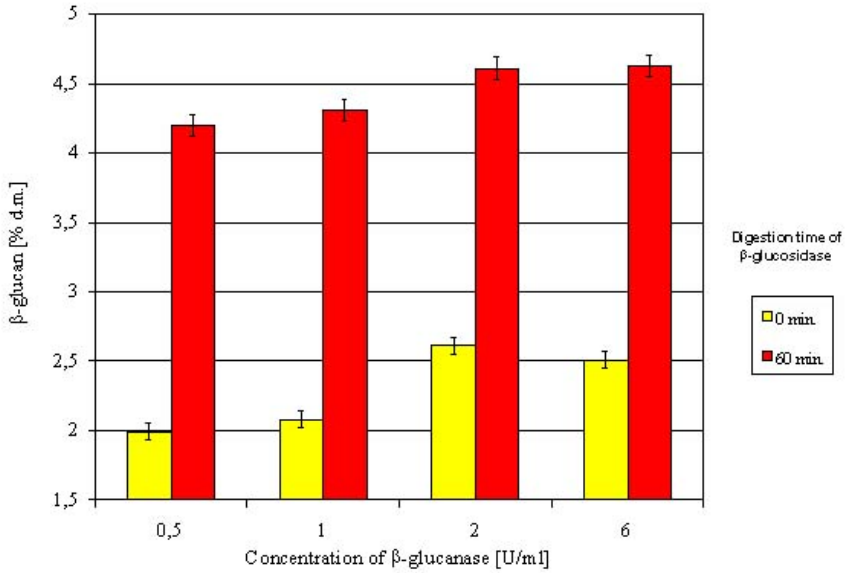


Figure 6. The effect of beta-glucanase concentration (hydrolysis time 180 min.) and beta-glucosidase (6U/ml) digestion time on beta-glucan content in whole barley grain of Nagrad variety



The results of the model experiment shows that due to their non-specific activity, the glucanolytic enzymes applied are likely to be partially responsible for cereal starch hydrolysis. The presence of starch hydrolysis products interferes in beta-glucan analysis, increases the result of determination. This was evidenced in the tests performed on whole barley grain. To demonstrate this effect, thermostable alpha-amylase Termamyl 120 L and alpha-amyloglucosidase were used. These enzymes did not cause hydrolysis of commercial beta-glucan preparation. Furthermore, the application of higher temperature during hydrolysis using thermostable alpha-amylase allowed for inactivation of native enzymes in the grains. Because of different level of cereal grain enzymatic activity, which depends mainly on climatic conditions during vegetation and harvesting, inactivation of endogenous enzymes appears to be a relevant.

Figure 2 presents a finally developed procedure for determining beta-glucan content in cereal material, including a starch elimination stage. Cereal material was ground to the granulation of < 250 µm, so the enzymes had an easier access to the hydrolyzed substrate. Thermostable alpha-amylase and alpha-amyloglucosidase were used for starch hydrolysis. The liberated glucose was removed by double washing of the sediment with 80% (v/v) ethyl alcohol. Ethyl alcohol caused also solubilisation of native sugars, dehydration and sedimentation of beta-glucan extracted during starch hydrolysis. Additional third washing of the sediment with the alcohol clearly decrease glucose level in a sample after enzymatic starch hydrolysis. In order to hydrolyze beta-glucan to glucose and glucooligosaccharides, beta-glucanase was added to centrifuged sediment. Beta-glucosidase was added to breakdown glucooligosaccharides to free glucose. Joint activity of beta-glucanase and beta-glucosidase caused decomposition of glucooligosaccharides into free glucose. This was confirmed by HPLC test results (Table 1). Application of those enzymes allowed for hydrolysis of beta-glucan of dehulled barley grain, to free glucose with the yield of 97%. There were remained only 3% of unhydrolysed glucooligosaccharides, including 1% of cellobiose and 2% of cellotriose. The content of the remnant glucose was determined with a modified DNS method.

Table 1. The effect of enzymatic hydrolysis of starch and beta-glucan determined by HPLC and DNS methods on the content of glucose and glucooligosaccharides in whole barley grain of Nagrad variety

Enzymatic hydrolysis Product	1 + 2		1 + 2 + 3		1 + 2 + 3 + 4	
	HPLC	DNS	HPLC	DNS	HPLC	DNS
Glucose and glucooligosaccharides in total [µg/ml]. including [%]:	116	140	1384	854	1509	1392
- glucose	100	100	52	100	97	100
- DP 2	0	-	5	-	1	-
- DP 3	0	-	8	-	2	-
- DP 4	0	-	3	-	0	-
- DP 5 and > DP 5	0	-	32	-	0	-

1 - alpha-amylase (Termamyl 120L)
2- alpha-amyloglucosidase
3 - beta-glucanase
4 - beta-glucosidase

Table 2 presents the results of beta-glucan determination in cereal grain and products. The highest beta-glucan content was observed for barley grain of Nagrad variety, especially for dehulled grain (4.87% ± 0.26). Beta-glucan content in commercial barley flakes was at the level of beta-glucan content in whole barley grain. Similarly, dehulled oats grain of Ułan variety (2.60% ± 0.25) presented higher beta-glucan content than whole oats grain (1.78% ± 0.44). Commercial oats bran were characterized by considerably higher content of this component (4.07% ± 0.26). The content of beta-glucan in rye grain of Dańkowskie Złote variety was 2.89% ± 0.20 whereas in triticale grain of Bogo variety 1.58% ± 0.46. Wheat grain results were in between of the two above (2.00% ± 0.23).

Table 2. The content of beta-glucan in cereal and selected cereal products

Cereal material	beta-glucan content [% dm]	Standard deviation	Coefficient of variation (%)	Confidence interval
Nagrad barley (whole grain)	4.62	0.10	2.2	0.30
Nagrad barley (dehulled grain)	4.87	0.09	1.7	0.26
Commercial barley flakes	4.53	0.09	1.9	0.26
Ułan oats (whole grain)	1.78	0.15	8.2	0.44
Ułan oats (dehulled grain)	2.60	0.08	3.2	0.25
Commercial oats bran	4.07	0.09	2.1	0.26
Dańkowskie Złote rye	2.89	0.07	2.3	0.20
Henika wheat	2.00	0.08	3.7	0.23
Bogo triticale	1.58	0.15	9.7	0.46

Statistical analysis of the results proved that as far as precision and reproducibility are concerned, the method described herein does not differ from other enzymatic methods of beta-glucan determination [1,7]. Standard

variation of the method was 0.10 while the coefficient of variation – 3.1%. The small differences between the results of beta-glucan determination obtained by other authors may result from the usage of various determination methods [1,4,6,7].

It is difficult to compare the results obtained in this work with the data from other countries because beta-glucan content in cereal grain is significantly affected by environmental factors, particularly by climatic, genetic and agricultural conditions. The tests were conducted for single varieties only and that is why it is impossible to draw general conclusions on beta-glucan content in cereal and cereal products.

The assessment of the above method of determining beta-glucan content in cereal and cereal products was performed by comparing the results obtained for nine samples of domestic barley varieties with McCleary and Codd method [7], recommended by ICC and AOAC (Table 3). This stage of the research was a part of grant financed by the State Committee for Scientific Research (KBN).

Beta-glucan content in barley samples determined by discussed method (DNS) (Table 3) varied from 3.85 to 4.65% dm and was lower than the results obtained by ICC method by an average of 15% (4.14-5.13% dm). However, the results let us claim that the method in question is characterized by a similar precision and reproducibility as ICC method, which is evidenced by similar values of standard deviation, precision and coefficient of variation. The method is relatively cheap to apply and can be used in laboratory with typical equipment. Its only disadvantage is long time of analyses, although other enzymatic methods are almost equally time-consuming [1].

Table 3. The content of beta-glucan determined by ICC and DNS methods in barley samples

Samples of barley	Beta-glucan [% dm]	
	ICC Standard 166	DNS
1.	4.34	3.94
2.	4.14	3.47
3.	4.56	3.92
4.	4.70	3.87
5.	4.21	4.01
6.	4.43	4.03
7.	4.65	3.85
8.	5.13	4.64
9.	4.85	3.79
Statistical parameters	ICC Standard 166	DNS
Average	4.56	3.95
Range	0.99	1.17
Max. value	5.13	4.64
Min. value	4.14	3.47
Method standard deviation	0.09	0.11
Method precision	4.56± 0.26	3.95± 0.30
Coefficient of variation [%]	1.97	2.85

CONCLUSIONS

1. Beta-glucanase (EC 3.2.1.6) and beta-glucosidase (EC 3.2.1.21) made by Fluka caused a partial decomposition of starch contained in cereal material. This requires an initial elimination of starch by application of alpha-amylase (EC 3.2.1.1) Termamyl 120 L from Novo and alpha-amyloglucosidase (EC 3.2.1.3) of BDH Biochemicals. These two enzymes do not adversely affect commercial preparation of beta-glucan.
2. Optimal working conditions (time and concentration) of the enzymes inducing beta-glucan hydrolysis were established. Hydrolysis time for beta-glucanase is 180 min at 2 U/ml concentration while for beta-glucosidase it is 60 min at 6 U/ml concentration.
3. Precision and reproducibility of discussed method of beta-glucan determination in cereal and cereal products is close to the standard and comparable to one recommended by ICC.

4. The performed tests let us obtain determinations of beta-glucan content which are free from errors caused by the application of non-specific and low purity enzymes, although the results obtained were lower than by ICC method by approximately 15%.

REFERENCES

1. Aman, P., Graham, H.: 1987. Analysis of total and insoluble mixed-linked (1→3),(1→4) beta-D-glucan in barley and oats. J. Agric. Food Chem., 35, 704-709.
2. Asp, N. G., Johansson, C. G., Hallmer, H., Siljestrom, M.: 1983. Rapid enzymatic assay of insoluble and soluble dietary fiber. J. Agric. Food Chem., 31, 476-482.
3. Ganssmann, W.: 1993. Beta-Glucan im Hafer und seine physiologische Wirkung [Beta-Glucan in oats and its physiological activity]. Getreide Mehl.u.Brot 47, 47-52 [in German].
4. Ganssmann, W.: 1994. Beta-Glucangehalte in deutschen Hefersorten und Laborversuche zur Anreicherung von beta-Glucan und Gesamt-Ballaststoffen [The beta-glucan content in German oat varieties. Laboratory investigation of possibilities to enrich oat products in beta-glucan and dietary fiber]. Getreide Mehl.u.Brot 48, 45-49 [in German].
5. Gąsiorowski, H. (red.): 1995. Owies: Chemia i technologia [Oats: Chemistry and Technology]. PWRiL Poznań [in Polish].
6. Henry, N. J.: 1987. Pentosan and (1→3), (1→4)-beta-glucan concentrations in endosperm and wholegrain of wheat, barley, oats and rye. J. Cereal Sci., 6, 253-258.
7. Mc Cleary, B. V., Cood, R.: 1991. Measurement of (1→3), (1→4)-beta-D-glucan in barley and oats: a streamlined enzymic procedure. J. Sci. Food Agric., 55, 303-312.
8. Michniewicz, J., Gąsiorowski, H.: 1994. Beta-Glukany zbóż - ich rola w przemyśle i żywieniu człowieka [Beta-Glucans in grain - their role in industry and human nutrition]. Postępy Nauk Rolniczych, 1/94, 40-49 [in Polish]
9. Michniewicz, J.: 1995. Inne polisacharydy nieskrobiowe [Non-starch polysaccharides]. s.62-68. W: Owies: Chemia i technologia [In: Oats: Chemistry and Technology]. (red. H. Gąsiorowski), PWRiL Poznań [in Polish].
10. Southgate, D.A.T.: 1991. Determination of food carbohydrates. Elsevier Sci. Publ., Barking U. K.

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