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KRYSTYNA J. CHRAPKOWSKA

DECOMPOSITION OF FRACTIONATED RYE BRAN BY CELLULASE COMPLEXES OF VARIOUS STRAINS OF THE FUNGUS *ARMILLARIELLA MELLEA* (FR. EX. WAHL) P. KARSTEN

Institute of Plant Food Technology, Agricultural Academy, Poznań

Key words: fractionation of rye bran, cellulase complexes, *Armillariella mellea*.

The fractionation of rye bran, done on a type DMK 0.81-4M DDR laboratory sifter, provided bran fractions with different contents of proteins, starch, cellulose and sugars. As a result of treatment of the bran fractions and unfractionated bran with cellulase complexes of various strains of the fungus *Armillariella mellea*, a higher degree of protein extraction from the fractions' aleuronic layer with a simultaneous higher degree of cellulose hydrolysis and reducing sugars increment as compared to the values for unfractionated bran, were obtained.

INTRODUCTION

The positive results obtained by Chrapkowska [1] in her work on the total extraction of proteins from the aleuronic layer of rye grain bran by the successive action of cellulolytic and proteolytic enzymes, as well as the growing interest of scientists in rye [6] prompted the launching of the present research. The objectives of the study were as follows:

1. Fractionation of rye bran on a laboratory sifter type DMK 0.81-4M DDR.
2. Chemical characteristic of whole bran and of fractions thereof.
3. Examination of the effect of cellulase complexes of various strains of the fungus *Armillariella mellea* (fr. ex. Wahl) P. Karsten on the degree of enzymatic decomposition of cellulose and hemicellulose in unfractionated and fractionated bran through the determination of:

— the degree of protein extraction from the aleuronic layer of unfractionated and fractionated bran,

— the degree of decomposition of the aleuronic layer cellular membrane and of the simultaneous increase of reducing sugars as glucose.

EXPERIMENTAL

MATERIAL

The rye bran taken for experiments came from Grain Mill No. 8 in Poznań (Regional Grain-Milling Industry Enterprise based in Poznań). The separation of bran was performed with a laboratory sifter (type DMK 0.81-4M DDR) and the material divided into fractions; the results of the separation are given in Figure. In view of the quantitative differences

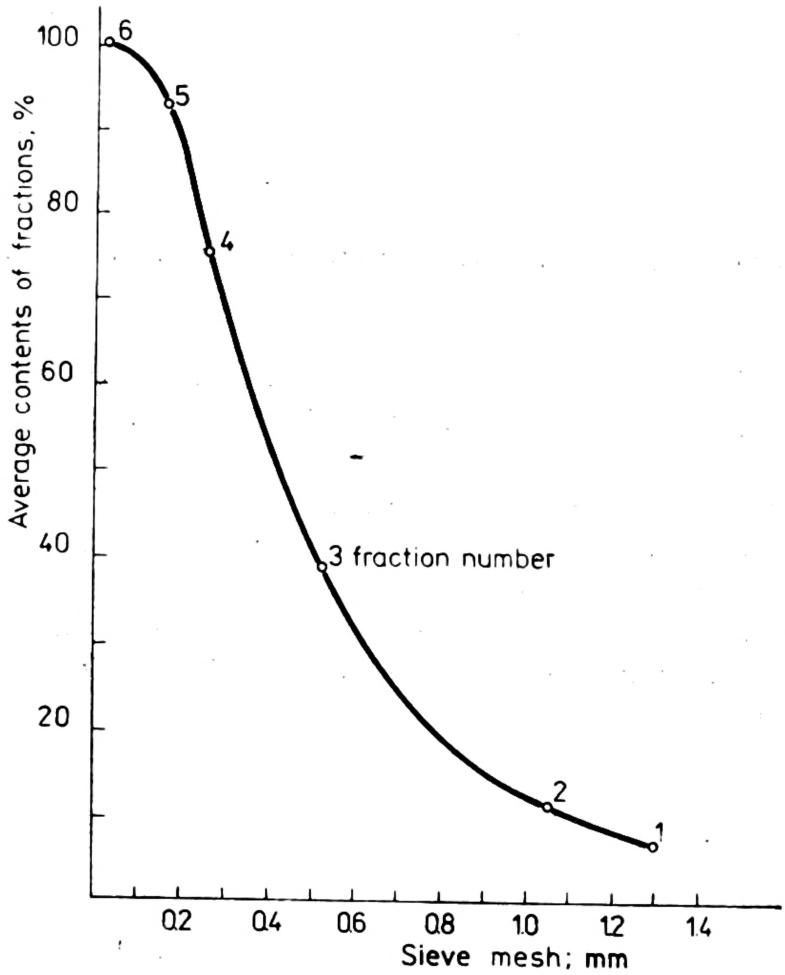

Fig. Mean shares of rye bran fractions obtained in 10 repetitions of separation on a laboratory sifter type DMK 0.81-4M DDR (bran portion for separation = 200 g)

Table 1. Chemical characteristics of whole bran and its fractions in dry matter, %

Whole bran and its fractions	Dry matter	Total protein (N × 6.25)	Reducing sugars as glucose	Cellulose	Starch
Whole bran	88.80	16.04	2.38	6.17	30.04
Fraction no.: 1	88.89	15.71	1.82	6.81	21.07
2	89.10	16.40	1.60	6.71	22.31
3	90.01	16.54	1.70	6.58	29.63
4	88.90	16.82	2.34	6.33	32.67
5	89.64	15.11	2.60	5.61	36.95
6	90.52	13.30	2.04	2.74	55.65

between the given fractions in the various repetitions, mean percentual contributions of the various fractions were calculated and the results of the study converted accordingly. The chemical characteristic of bran and its fractions is given in Tab. 1. Apart from data for whole bran also determined were: fat in the amount of 4.03%, total ash — 4.12%, and ash insoluble in 10% HCl — 0.09%.

The cellusases producers were three strains (1, ZO-11 and Aśw.-26) of the fungus *Armillariella mellea* (Fr. ex. Wahl) obtained from the Institute of Forest Protection of the Scientific Teaching Center of Forest Phytopathology, Agricultural Academy, Poznań. A detailed description of the strains can be found in Chrapkowska's work [2]. The reagents came from the trade and manufacturing company "Polskie Odczynniki Chemiczne" (Polish Chemical Reagents) based in Gliwice.

METHODS

The dry mass of bran and cellulose was determined by the method of drying, nitrogen — by Kjeldahl's method, reducing sugars — by the method of Samogyi-Nelson [5], cellulose by the method of Scharrer-Kurschner according to Kamer and Glinkel [3], fats according to Soxhelet, starch — by the method of Baumann-Grossfeld according to Pawełkiewicz [4] and ash — by incineration at 550°C for 6 h. Active crude cellulases preparations were obtained from all three strains of *A. mellea* by the method of submerged culture [2]. The method of determining the cellulolytic activity of complexes is given in [2]. The enzymatic hydrolysis of bran and protein extraction was done by the method of Chrapkowska [1].

EXPERIMENT

The post-culture fluids obtained from the cultivation of three strains of *A. mellea* with cellulolytic activity described in [2] were taken as the crude cellulolytic preparations; rye bran and its fractions were treated directly with the preparations according to the method of Chrapkowska [1]. After the enzymatic hydrolysis of bran and its fractions, the content of proteins and sugars was determined in the clear fluids, and cellulose in the sediments. The results are shown in Tab. 2.

RESULTS AND DISCUSSION

Rye bran was fractionated on a laboratory sifter type DMK 0.81-4M DDR, being thereby divided into fractions with different contents of protein, starch, cellulose and monosaccharides. An exact analysis of bran

Table 2. Results of hydrolytic direct effect of cellulase complexes in selected strains of fungus *Armillariella melea* (Fr. ex. Wahl) P. Karsten on whole bran and its fractions

Mushroom strain	Rye bran and its fractions	Protein (N × 6.25) extractable after hydrolysis in ratio of initial contents %	Increment of reducing sugars as glucose after hydrolysis %	Decrement of cellulose after hydrolysis %	Reducing sugars in ratio of cellulose per 100 g dry matter of bran sample %
Whole bran 1	Whole bran	22.88	12.02	5.98	43.21
	Fraction No.:				
	1	21.73	15.99	7.81	31.00
	2	22.48	20.56	8.91	28.75
	3	22.32	33.67	9.44	34.57
	4	22.02	12.05	4.64	41.42
	5	22.94	9.14	3.90	50.68
	6	35.87	7.60	3.58	80.11
Average sum total of fractions:		24.22	15.41	6.80	44.42
Zo-11	Whole bran	2036	6.39	3.70	41.04
	Fraction No.:				
	1	19.61	3.96	5.52	27.78
	2	18.94	5.81	5.08	25.23
	3	19.90	7.40	5.26	27.78
	4	22.58	16.92	5.69	43.22
	5	21.46	14.93	5.33	53.37
	6	32.88	14.80	5.22	85.47
Average sum total of fractions:		22.25	11.40	5.36	43.81
AśW-26	Whole bran	26.14	15.17	6.61	44.42
	Fraction No.:				
	1	24.90	13.79	7.37	30.41
	2	27.35	36.81	11.21	32.62
	3	27.39	16.63	7.74	30.17
	4	28.10	15.81	5.24	42.81
	5	25.76	16.70	7.18	54.19
	6	47.08	13.87	5.15	84.78
Average sum total of fractions:		29.33	18.24	7.59	45.83

separation is given in Figure. The obtained bran fractions and whole bran were subjected to the enzymatic activity of cellulases complexes obtained from three strains of the fungus *Armillariella mellea*. The results are collected in Tab. 2.

According to the results presented in Figure and Tab. 1, the fractionation of rye bran makes it possible to separate bran fractions characterized by various contents of proteins, starch, cellulose and sugars

different from those in unfractionated bran. The various content of these components in bran fractions is caused not only by the specific separation of the endospermic part of bran, but mainly by the differentiated fractionation of proteins and cellulose of the bran's aleuronic layer. This enables various technological utilizations of the fractions in food and fodder production. Moreover, the treatment of the obtained bran fractions with cellulase complexes of *A. mellea*, compared with an analogous treatment of unfractionated bran, gave encouraging results, as we can see in Tab. 2. The highest extraction of proteins from unfractionated bran — 26.14% — was obtained in the case of treatment with the cellulase complex of strain AśW-26; strain 1 extracted 22.8% and strain ZO-11 — 20.36% of the proteins. A much higher proportion of proteins was extracted from aleuronic layer cells by acting with cellulase complexes on fractionated bran, as compared with a similar treatment of unfractionated bran. Out of all the treatments of fractions with the three strains of *A. mellea*, the best results were obtained in the case of fraction No. 6. The greatest protein extraction, 47.08%, occurred in this fraction during treatment with cellulases obtained from strain AśW-26; strain 1 extracted 35.87% and strain ZO-11 — 32.88% of the proteins — the second and third best results. This indicates that fraction No. 6, containing the least amount of cellulose prior to hydrolysis (2.7% — of. Tab. 1) contained the highest amount of proteins liberated from the cells. Fraction No. 6 had the least protein content (13.30% — cf. Table 1); it is composed of the smallest particles, as compared with the remaining fractions, and for this reason its proteins were extracted more easily during enzymatic hydrolysis, although, as we know, the cellulose of aleuronic layer cells is more resistant to hydrolysis. The cellulases of strain AśW-26 exhibited also the greatest activity in most of the bran fractions (Tab. 2). The cellulase complexes obtained from all the strains acting on some of the bran fractions extracted more proteins than when acting on unifractionated bran.

On the basis of earlier work 1 on the application of proteolytic („Pronase") and cellulolytic enzymes in total extraction of proteins from the aleuronic layer of unfractionated rye bran, we may conclude that by treating fractionated bran with cellulases it is possible to reduce both the pronase dose and the time of reaction during the separation of the aleuronic layer from the endospermic part of bran, obtaining at the same time maximum effectiveness of bran hydrolysis by cellulases.

Also cellulose losses after hydrolysis were differentiated and higher in fractionated bran than in the case of unfractionated bran hydrolysis. The greatest cellulose losses, amounting to 11.21%, were recorded during the treatment of bran fraction No. 2 with cellulases of strain AśW-26, and in the case of strain 1 acting on fractions No. 3 (9.44%) and No. 2 (8.91%) (cf. Tab. 2).

The increments of reducing sugars as glucose obtained from the hydro-

lysis of cellulose and hemicelluloses in fractionated bran were also usually higher than in the case of unfractionated bran. The greatest increments of sugars were obtained in the treatment of bran fraction No. 2 with cellulases from strain AśW-26, namely 36.81%. The next best results were recorded when cellulases of strain 1 acted on fractions No. 3 (33.67%) and No. 2 (20.56%) and when fraction No. 4 was treated with cellulases of strain ZO-11 (16.92%). It must be mentioned here that of all the cellulases used, only that of strain AśW-26 hydrolysed to monosaccharides cellulose and hemicelluloses jointly, in all fractions and to a very high degree (Tab. 2).

Calculations of the ratio of reducing sugars to cellulose content in 100 g dry matter of samle demonstrated that the values are also differentiated and in the case of fractionated bran often very high in comparison to the values for unfractionated bran (Tab. 2). The highest values were obtained here for all three strains acting on fractions No. 6 and 5. In the case of fraction No. 6, the obtained values were (in order of decreasing magnitude): for strain ZO-11 — 85.47%, for AśW-26 — 84.78%, for strain 1 — 80.11%. Thea nalogous values for fraction No. 5 were: for strain AśW-26 — 54.19%, for ZO-11 — 53.37%, for strain 1 — 50.68%. In order to demonstrate the higher susceptibility of fractionated bran to the hydrolytic action of various strains of *A. mellea*, as compared to the results of hydrolysis of unfractionated bran, mean values for each fraction were calculated on the basis of the sums of results obtained for the distinguished fractions (cf. Tab. 2). As we see, the mean values for all fractions exceed the analogous values obtained for unfractionated bran as regards protein extractability, increment of reducing sugars, cellulose loss, and the ratio of reducing sugars increment to cellulose content in 100 g dry matter of samples.

CONCLUSIONS

1. The fractionation of rye bran on a laboratory sifter type DMK 0.81-4M DDR leads to the separation of bran fractions differing as to the content of protein, starch, cellulose and reducing sugars as glucose. This creates the possibility of different technological processing and utilization as both food and fodder.

2. The hydrolytic action of cellulase complexes of the fugus *Armillariella mellea* (Fr. ex. Wahl) P. Karsten on rye bran fractions leads to a greater extraction of proteins from the aleuronic layer and of reducing sugars as glucose in the decomposition of cellulose than in the hydrolysis of unfractionated bran.

3. The greatest amount of proteins was extracted from the aleuronic layer by acting with cellulase complexes of all three strains on bran

fraction No. 6 (overtails), the one that is most poor in cellulose in comparison with whole bran and with the remaining fractions.

4. Of the three strains of *A. mellea* that were studied, the most active cellulase complexes were produced by AśW-26, both as regards their effect on unfractionated and on fractionated bran.

5. The increment of reducing sugars obtained as a result of treating bran fractions with cellulases was higher in cellulose-rich fractions as compared to the reducing sugars increment obtained in the hydrolysis of unfractionated bran cellulose.

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Author address: 60-624 Poznań, Wojska Polskiego 31.

K. J. *Chrapkowska*

ROZKŁAD FRAKCJONOWANYCH OTRĄB ZIARNA ŻYTA PRZEZ KOMPLEKSY CELULAZ RÓŻNYCH SZCZEPÓW GRZYBA *ARMILLARIELLA MELLEA* (FR. EX. WAHL) P. KARSTEN.

Instytut Technologii Żywności Pochodzenia Roślinnego, Akademia Rolnicza, Poznań

S t r e s z c z e n i e

Przeprowadzono frakcjonowanie otrąb żytnich na odsiewaczu laboratoryjnym typ DMK 0.81-4M DDR. Dzięki temu zabiegowi uzyskano frakcje otrąb o zróżnicowanej zawartości białek, skrobi, celulozy i cukrów redukujących. Również udział procentowy poszczególnych frakcji był zróżnicowany (rys. 1). Charakterystyka chemiczna frakcji otrąb wykazała różną zawartość poszczególnych składników: białka, skrobi, celulozy i cukrów (tab. 1), co stwarza możliwości różnego ich technologicznego zastosowania i wykorzystania zarówno na cele żywieniowe człowieka, jak i paszowe zwierząt.

W wyniku hydrolitycznego działania kompleksów celulaz różnych szczepów grzyba *Armillariella mellea* (Fr. ex. Wahl) P. Karsten na frakcje otrąb żytnich uzyskano wyższy stopień wydobycia białek z warstwy aleuronowej oraz większy rozkład celu-

lozy przy równoczesnym większym przyroście cukrów redukujących, w porównaniu ich z wartościami dla otrąb niefrakcjonowanych (tab. 2).

Najwyższy stopień uwalniania białek warstwy aleuronowej otrzymano pod wpływem działania kompleksów celulaz wszystkich trzech szczepów na frakcje otrąb nr 6 (zlot), (tab. 2) zawierającą najniższy procentowy udział celulozy w porównaniu z otrębami całymi i pozostałymi frakcjami (tab. 1).

Z przebadanych trzech szczepów grzyba *Armillariella mellea* najaktywniejszy był szczep AśW-26 i to zarówno w działaniu na otręby niefrakcjonowane, jak i ich frakcje.