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AMINOACID COMPOSITION OF PROTEINS OF ENDOSPERM FRACTION AND ALEURONIC LAYERS BARLEY AND OATS BRANS

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Key words: endosperm, aleuronic layers, oats bran, proteolytic enzymes, cellulolytic enzymes, aminoacid composition.

Proteins from the endosperm fraction and the aleuronic layers in barley and oats brans were isolated by means of proteolitic enzymes. The isolated aleuronic layer was then decomposed with a set of cellulases derived from *Armillariella mellea* (Fr. ex. Wahl.) P. Karsten fungus. It was also subjected to release of proteins. Aminoacid composition of endospermic and aleuronic fractions was determined. Chemical indices of the above were formulated.

Cereal fodder deriving from brans contains significant guantities of cellulose, mineral salt and other nutrients. Some of them locate in the aleuronic layer in which the cellular membrane consists of cellulose. Since neither pigs nor poultry digest cellulose the nutrients in the aluronic layer are not fully assimilated by these animals.

Decomposition of the cellular cellulose membranes of the cells in the aleuronic layer makes their content (of the cells) available. It is rather quite nutritious.

The following paper was aimed at the characterising barley and oats endosperm fractions and their aleuronic layers.

MATERIALS

Raw materials (1977) consisted of barley and oats brans from one of the Polish cereal processing plants (Wągrowiec). The parameters of the brans are given in Table 1 [3].

Enzymes used in the analyses were as follows:

1. Proteolytic: Pronase B-grade, Calbiochem. San Diego, California 92112.

2. Cellulolytic: Two specific sets of cellulases obtained by the authors by cultivation of *Armillariella mellea*, an edible fungus. Earlier papers [2, 3, 4] provide cultivation conditions, raw preparations, and detailed characteristics of enzymatic activity of the complexes.

Table 1. Chamical composition of barley and oats brans; % per dry mass unit

Components	Brans	
	barley	oats
Dry mass	92.42	91.29
Total protein (N × 6.25)	16.83	14.79
Reducing sugars (as glucose)	2.59	1.95
Cellulose	10.46	13.51
Fats	4.24	8.17
Starch	45.66	33.61
Ash	4.17	3.38

METHODOLOGY

Brans of barley and oats were subjected to an analysis aimed at isolating the aleuronic layer and characterising the proteins present in the endospermic and aleuronic parts of the brans. The isolation of aleuronic layers was performed by treating ground brans of the investigated cereals with proteolytic enzymes, which was followed by application of specific cellulases to the isolated aleuronic layer. The experimentation was performed according to the following sequence:

25 g ground brans
was moisturized with 20 ml Tris (pH 7.4) buffer containing 20 mg proteases
↓
incubation: 48 hrs, 37°C
↓
centrifuging ——→ Extract containing endospermic protein (in which aminoacid composition and protein level were determined)
↓
sediment
including the isolated aleuronic layer (it was added with 25 ml cellulases specific preparation at pH 5.0)
↓
incubation: 36 hrs, 37°C
↓
inactivation of enzymes by heating for 10 min. at 90°C
↓
centrifuging
↙ ↓ ↘
remaining — Extract containing the aleuronic proteins (in which aminoacid composition and protein contents were determined).
sedimentation

The brans from barley and oats were also subjected to direct digestion with specific cellulases under the same conditions in which the aleuronic layer isolated from brans was treated. The extracts obtained in effect of treatment with proteolytic and cellulolytic enzymes were examined as to their protein contents and aminoacid composition while in the remaining sediments the level of cellulose was determined. In the course of the determining procedures the following methods were applied: cellulose — Scharrer-Kürschner after Kamer et al [7], reducing sugars — after Somogyi-Nelson [11] — these results were reported in an earlier study [3], nitrogen — after Kjeldahl, aminoacid composition — after Spackman et. al [12] using a Beckman Multichron analyzer. On the basis of the analysis of proteins in aminoacids of the endosperm and the aleuronic layer the chemical score of these proteins in ratio of the standard protein in hen egg whites was calculated. This stands for the index of the limiting aminoacid WAO — after Block et al [1].

RESULTS

Table 2 shows quantities of protein observed in the endospermic part and in the aleuronic layer of the brans as well as in the barley and oats brans treated anzymatically [3].

Table 2. Protein contents in barley and oats brans and in the endospermic and aleuronic fractions of the brans

Raw material	Protein determined in extract after degestion with (%)		
	proteases	cellulases	proteases and cellulases
Barley bran	30.53	45.77	98.18
Oats bran	27.54	30.15	82.54

Enzymatic hydrolysis of the analyzed cereals showed a very high degree of protein release. Successively applied treatments with proteases and cellulases provided best results. It was observed that barly bran is a more susceptible material to enzymatic hydrolysis since it provides more protein in the process as compared to oats bran.

Aminoacid composition of the obtained proteins was analysed. Results are given in Tables 3 and 4. The qualitative composition of aminoacids in all the analyzed proteins is the same.

Table 5 presents contents of certain essential aminoacids in the analyzed proteins. The hen egg-white aminoacid composition was used for comprison and the chemical score for aminoacids was calculated.

Table 3. Contents of aminoacids in proteins released from barley and oats brans by treatment with proteases

Aminoacids	Content of aminoacids in mg per 1 g total protein in brans from		Percentage of aminoacids in ratio of aminoacids determined in brans from	
	barley	oats	barley	oats
Lysine	46.6	66.3	5.3	7.5
Histidine	7.4	7.7	0.8	0.9
Arginine	9.7	17.9	1.1	2.0
Aspartic acid	30.9	107.1	3.5	12.2
Treonine	14.3	12.8	1.6	1.5
Serine	14.3	35.7	1.6	4.0
Glutamic acid	139.2	119.9	15.8	13.6
Proline	91.6	45.9	10.4	5.2
Glycine	49.4	71.4	5.6	8.1
Alanine	60.1	66.3	6.8	7.5
1/2 Cystine	21.8	25.5	2.5	2.9
Valine	51.2	102.0	5.8	11.6
Methionine	20.4	25.5	2.3	2.9
Isoleucine	39.5	12.8	4.5	1.5
Leucine	69.1	30.6	7.9	3.5
Tyrosine	181.4	71.4	20.6	8.1
Phenyloalanine	33.0	61.2	3.8	7.0
Total	879.9	880.0	99.9	100.0

Deriving from the above data, aminoacids limiting the biological value of the analyzed proteins were determined.

Results of proteolytic and cellulolytic enzyme treatmens of barley and oats brans make it possible to surmise that proteins resulting from the protease activity derive from endospermic parts of bran. Cellulases primarily release proteins from the aleuronic cells. Successive treatment with both of the enzymatic complexes renders it possible to solubilize nearly the entire volume of protein from barley and oats brans. The barley protein consists of albumin (15%), globulin (10 to 20%) prolamin (35%), and glutelin (ca 35%). In terms of nutrition albumins and globulins are more valuable. They are located in the external or outer layers of the kernel that is to say, in the aleuronic area. Their value derives from the fact that the levels of lysine and other aminoacids are quite substantantial in them. Barley prolamins are in the endospermic part of the kernal and are called hordein Their nutritional value is not very high since they contain much proline and glutamic acid while being short of lysine, methionine, treonine and histidine [6].

Table 3 presents the aminoacid composition of proteins in the endospermic part of barley and oats brans. Table 4 provides the data on their

Table 4. Contents of aminoacids in proteins released in effect of cellulases of *Armillariella mellea* (fr. wx. Wahl) P. Karsten on the aleuronic layers in barley and oats brans isolated with proteases

Aminoacids	Content of aminoacids in mg per 1 g total protein in brans from		Percentage of aminoacids in ratio of aminoacids determined in brans from	
	barley	oats	barley	oats
Lysine	75.0	68.1	8.5	7.9
Histidine	9.0	4.1	1.0	0.5
Arginine	31.4	30.3	3.6	3.5
Aspartic acid	134.3	160.7	15.7	18.6
Treonine	39.2	59.9	4.5	6.9
Serine	35.8	54.1	4.1	6.3
Glutamic acid	139.9	149.2	16.0	17.3
Proline	15.7	11.5	1.8	1.3
Glycine	67.2	72.2	7.6	8.4
Alanine	97.4	87.7	11.1	10.2
1/2 Cystine	23.6	traces	2.3	—
Valine	52.6	49.2	6.0	5.7
Methionine	31.5	13.1	3.5	1.5
Isoleucine	25.8	27.9	2.9	3.2
Leucine	66.1	61.5	7.5	7.1
Tyrosine	17.9	8.2	2.0	1.0
Phenyloalanine	16.8	4.9	1.9	0.6
Total	879.2	862.6	100.0	100.0

Table 5. Comparison of levels of certain exogenous aminoacids in egg-white protein (after FAO/WHO 1973/5) and in proteins from brans of the analyzed cereals; mg/g protein (N × 6.25) and the chemical score

Aminoacids	Egg-white protein	Barley aleuronic	Barley endosperm-mic protein	aleuronic	Oats endospermic protein
Isoleucine	66	39	60	42	19
Leucine	88	75	79	70	35
Lysine	64	117	73	105	104
Methionina + cystine	55	100	77	24	93
Phenyloalanine + tyrosine	100	34	214	13	133
Treonine	51	77	28	117	25
Valine	73	72	70	67	140

aleuronic layers. Comparing the differences in the aminoacid compositions of the two fractions in berley bran, we may say that there are higher levels of lysine treonine, valine and methionine in aleuronic rather than in the endosperm proteins. Leucine and cystine keep same levels but there

is less tyrosine and phenylalanine. The above results indicate that proteins from the analyzed cereals are highly nutritional, particularly those deriving from their aleuronic layers.

Analysis of differences in the composition of essential aminoacids from endospermic and aleuronic fractions of oats bran shows increased levels of lysine, arginine, treonine and leucine, the other live essential aminoacids, including methionine, are more pronouncedly present in the endospermic protein.

Table 5 gives quantities of essential aminoacids in the analyzed protein fractions as well as levels of the same aminoacids in egg white. The level of egg white aminoacids was used as a standard in the comparative evaluation of the investigated proteins of barley and oats carried out on the basis of the aminoacids content and WAO chemical score. It turned out that in the oats endospermic proteins the limiting aminoacid is isoleucine while in barley it is treonine. This corresponds with the determined fact that barley hordeine contains low levels of this aminoacid. It was also found that in the aleuronic proteins of the two cereals phenyloalanine and tyrosine are the limiting aminoacids. This fact suggests certain similarities in the quantitative composition of aminoacids in the cereals particularly since treonine, which is the limiting aminoacid of barley endospermic proteins, has also a very low chemical score in endospermic proteins in oats.

The reported analyses indicate that brans from barley and oats can be enriched with protein components, which are not assimilated by non-ruminants, in the course of treatment with enzymes. Numerous works by Munck [8, 9, 10] and others concerning the nutritional value of proteins from barley and other cereals provide valuable information on the aminoacid composition, especially ofexogenous kind, and their digestibility. The author suggests that barley endospermic proteins are better digested by non-ruminants than the high-lysine aleuronic proteins. It seems that one of the essential reasons is the cellulose membrane in aleuronic cells. It constitutes a natural difficulty for animals with a single stomach which do not have a properly developed system of cellulases in their digestive tract.

CONCLUSIONS

In effect of the above analyses it was observed as follows:

1. Application of protases and cellulases to isolate the aleuronic layers in brans from barley and oats made it possible to determine the aminoacid composition of these as well as endospermic proteins.
2. Aleuronic proteins in barley and oats brans are richer in essential

aminoacids than the endospermic ones thus being more useful in nutrition.

3. Calculation of the chemical score in endospermic and in aleuronic proteins from two brans indicates superior nutritional value of the aleuronic type.

4. The chemical score of limiting aminoacids in the analyzed endospermic and aleuronic fractions of proteins suggests cartein similarities in the quantitative composition of aminoacids.

5. It is possible and purposeful to release aleuronic proteins from the brans for future use in nutrition of human beings and animals.

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SKŁAD AMINOKWASOWY BIAŁEK FRAKCJI BIELMOWEJ I WARSTWY ALEURONOWEJ OTRĄB JĘCZMIENIA I OWSA

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Streszczenie

Przeprowadzono badania nad charakterystyką białek frakcji bielmowej i warstwy aleuronowej otrąb jęczmienia i owsa. Warstwę aleuronową izolowano przez hydrolityczne działanie na część bielmową otrąb enzymami proteolitycznymi (pro-

nazą). W wyniku działania pronazy uwolniono 30,53% białek z części bielmowej otrąb jęczmiennych i 27,54% z otrąb owsianych. W wyniku analizy składu aminokwasowego białek bielmowych w otrębach z jęczmienia i owsa, a następnie obliczenie chemicznego wskaźnika wartości dla aminokwasów egzogennych występujących w nich okazało się, że najniższe wskaźniki chemical score mają dla białek bielmowych w jęczmieniu treoninę, w białkach bielmowych owsa histydynę oraz treoninę. Następnie na odizolowane warstwy aleuronowe obu rodzajów otrąb podziałano specyficznymi kompleksami celulaz grzyba *Armillariella mellea* (Fr. ex. Wahl) P. Karsten. Rozłożone w ten sposób celulozowe błony komórkowe tych warstw pozwoliły uwolnić znajdujące się tam białka. W wyniku sukcesywnego działania proteaz i celulaz uwolniono 98,18% białek otrąb jęczmiennych i 82,99% białek otrąb owsianych. W białkach aleuronowych obu badanych otrąb zbożowych aminokwasami ograniczającymi jest fenyloalanina i tyrozyna. Wyraźnie różnią się natomiast białka aleuronowe jęczmienia i owsa zawartością metioniny. W białkach izolowanych z warstwy aleuronowej jęczmienia jest czterokrotnie więcej tego aminokwasu niż w białkach aleuronowych otrąb owsianych. Stwierdzono wysokie wskaźniki chemical score dla lizyny w białkach z warstwy aleuronowej obu rodzajów badanych otrąb zbożowych, a także w białkach bielmowych tych otrąb.

Uzyskane w toku przeprowadzonych doświadczeń wyniki świadczą o wysokiej wartości żywieniowej białek badanych zbóż, zwłaszcza białek gromadzonych w warstwie aleuronowej jęczmienia oraz białek bielmowych jęczmienia i owsa. Białka pochodzące z warstwy aleuronowej otrąb owsianych mają mniejszą wartość żywieniową ze względu na niską zawartość metioniny.