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PRODUCTION OF OAT HYDROLYSATES WITH A LOW DEGREE OF STARCH SACCHARIFICATION

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The manuscript presents methodology of obtaining a hydrolysate from ground oat grain under laboratory conditions. The methods applied involved the application of the enzymatic hydrolysis of starch with the use of a thermostable amylolytic preparation Nervanaze T. Parameters of the hydrolysis process were determined by means of acidic inactivation that enables obtaining an oat hydrolysate with a glucose equivalent DE 5.5±0.5 and 4-5% of health-promoting β-glucans applicable mainly as fat replacers.

INTRODUCTION

Starch maltodextrins produced during partial hydrolysis of gelatinized starch, by means of acids permitted for use in the food industry and/or enzymes [Richter *et al*., 1976a,b; Roller, 1996; Bryjak, 1999], have found a number of industrial applications, namely in the production of a variety of new products, especially those with reduced content of fat with interesting organoleptic traits. Their presence in a product facilitates technological processes, contributes to the extension of shelf life (water absorption and binding capacities) and reduces calorific value, as well as prevents the occurrence of unfavorable phenomena (*e.g.* syneresis). Due to their properties, maltodextrins may be applied, *i.a.* as stabilizers, emulsifiers, raising agents, and fillers, thus contribute to the possibility of introducing new extended assortments of food products.

In the production of maltodextrins – preparations with a glucose equivalent DE below 20 – use is most often made of the following starches: potato, maize, wheat, rice and tapioca [Sawicka-Żukowska *et al*., 1999; Fortuna *et al*., 2000; Dokic-Baucal *et al*., 2004]. Products obtained as a result of the direct action of enzymatic preparations on starches are referred to as maltodextrins, syrups. In the case of the reported experiments, in which the enzymatic reaction was applied to meal of the ground whole oat grain, the resultant end product should be referred to as a hydrolysate: a preparation that may contain also other non-starch constituents (*e.g.* β-glucans), being of significance to chemical, physical or nutritional properties.

On the American market there are available preparations obtained upon hydrolysis of oat flour – Beta-Trim™ and Trim Choice, or those obtained from oat grain subjected to selective extraction – Nu-Trim, as well as those produced from the whole oat grain – OATRIM [Inglett & Newman, 1994]. Depending on the fraction of oat grain used for hydrolysis, the resultant preparations were characterized by various contents of β-glucans (3%, 5% and 10%), which has been reflected in names of respective hydrolysates, *i.e.* OATRIM 3, OATRIM 5 or OATRIM 10 [Anonymous, 1990]. The presence of β-glucans in hydrolysates results in an increase in the nutritional value, which in turn contributes to their wider application. Such hydrolysates are applied as texturizing agents, whereas due to the possibility of replacing fat they reduce the calorific value in confectionery and cake products, frozen desserts, dairy drinks, cheeses, dressings, processed meat products or salads [Anonymous, 2000; Sampaio *et al*., 2004; Zoulias *et al*., 2002; Konuklar *et al*., 2004; Volikakis *et al*., 2004; Gibiński *et al*., 2006; Worrasinchai *et al*., 2006; Walczycka *et al*., 2006].

Thus, the presented study was aimed at elaborating a methodology that would enable obtaining first ever in Poland hydrolysate preparation with a glucose equivalent DE of *ca*. 5.0 from grain of Polish cultivars of oat to be applied as a fat replacer in selected food products.

MATERIALS AND METHODS

The experimental material was commercial oat grain obtained from PZZ "Wieczysta" in Cracow, Poland. The grain was ground in a Cyclotec 1093 Sample Mill by Foss Tecator with a screen with mesh diameter of 0.5 mm.

Investigations involved: selecting the concentration of meal suspension; determining the method of enzyme inactivation; and determining the susceptibility of oat meal to the actions of an enzymatic preparation.

Laboratory analyses carried out in the study included: (1) determination of the enzymatic activity according to SKB (Sandstedt, Kneen, Blich) [Sandstedt *et al.*, 1939; Hagberg,

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1961]; (2) determination of a glucose equivalent (DE) according to Polish Standard [PN-EN ISO-5377: 2001]; (3) determination of the content of glucose oligomers G1-G8 with the HPLC method [Brookes & Griffin, 1987] in the investigated hydrolysate and in a commercial preparation OATRIM; (4) determination of glucose content according to Polish Standard [PN-EN ISO-5377: 2001]; and (5) determination of the content of β-glucans according to ICC Standard [Method no. 168].

Enzymatic hydrolysis was conducted with the use of thermostable bacterial α-amylase (*Bacillus licheniformis)* NERVANAZE-T (Ubichem) with the optimal activity at: temperature of 95-105°C, pH of 5.8-6.2, and preparation activity of 800-850 units/mL determined according to SKB. The process of hydrolysis was run in a bath with silicone oil Si 0026 by Scharlau Chemie SA as a medium. A water suspension of meal was stirred using an EUROSTAR agitator by IKA Labortechnik Werke, and after the hydrolysis it was centrifuged in a ROTANTA 46 R centrifuge by Hettich at 11,000×*g* (at a constant temperature of 20°C). The resultant hydrolysate was dried in a Ralpha 1-4 LSC freeze-dryer by Christ, and the dried preparation was ground and sieved in a kit FRITSCH Mortar Grinder PULVERISETTE 2 and FRITSCH Laborgeraetebau through 0.12 mm screen mesh (the scheme of the hydrolysis process is presented in Figure 1).

That methodology was elaborated based on Inglett's procedures [Inglett, 1990; Inglett & Newman, 1994], with some modifications, *i.e.*: with the application of a different enzymatic preparation and using acidic inactivation instead overheated water vapour.

In the chromatographic method used for determinations of oligomers, the prepared filtrate was injected onto an HPLC column (HPX 42A; 7.8 × 300 mm; Bio-Rad). The separation was conducted at a temperature of 85°C with water used as a mobile phase at a flow rate of 0.6 mL/min. Determinations were performed by means of an IR detector.

Analyses aimed at determining the content of glucose oligomers G1-G8 in the obtained hydrolysate and a commercial preparation OATRIM were carried out with the use of an OATRIM 5 Q preparation by Meyhall Chemical AG.

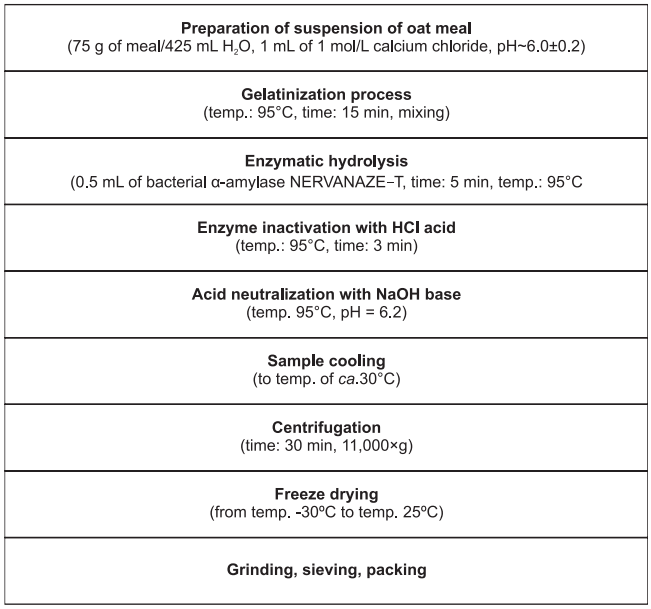

FIGURE 1. Scheme of obtaining an oat hydrolysate with saccharification degree (DE) of *ca*. 5 (laboratory method).

Results presented in Tables 2-5 are arithmetic means from ten measurements.

All results were verified with the Q-Dixon's test used for the rejection of results burdened with a gross error [Zgirski & Gondko, 1998]. For 10 measurements in series, all Q_A/Q_B values were lower than the value read out from a table, *i.e.* Q_{kr}=0.504, thus all the results obtained were accepted for interpretation.

The results obtained were subjected to a one-way analysis of variance to compute LSD, and interpreted with a posteriori Tukey's test [Łomnicki, 1999] at a significance level of α=0.05. Differences were found statistically significant (different letters) when the absolute difference of the mean values was higher than the Least Significant Difference (LSD); whereas no statistically significant differences were reported once the absolute difference of mean values was lower or equal to LSD (the same letters in tables).

RESULTS AND DISCUSSION

Selection of the concentration of oat meal suspension

Analyses were carried out in aqueous suspensions with oat meal concentrations of 10, 15 and 20%. Behaviour of 500-g aqueous suspension of the meal was observed during its heating (for 15 min) up to a temperature of 95°C [Gibiński & Pałasiński, 1991; Inglett & Newman, 1994] (optimum of oat starch gelatinization) as well as within the first 5 min since the administration of the enzymatic preparation and rising the temperature up to 100°C. Afterwards, the flask was cooled with cold water, its content was gently decanted to a beaker, and observations were made for the degree of suspension mixing and the condition of flasks interior (Table 1).

TABLE 1. Effect of the selection of starch suspension concentration on the course of the hydrolysis process.

Suspension concentration (%)	Evaluation of the course of hydrolysis
10	The suspension was well mixed; along with temperature increase the process of gelatinization proceeded evenly; enzyme addition caused a substantial decrease in suspension viscosity, its abrupt splashing on walls of the flask and necessity of decreased the frequency of an agitator revolutions; after cooling the sample preserved a homogenous structure and colour.
15	Until the beginning of the gelatinization process the sample was well mixed and homogenous, without lumps; since 90°C – an increase was observed in viscosity; a decrease in viscosity after enzyme addition did not cause the abrupt splashing of the contents of the flask; after cooling the sample preserved a homogenous structure and colour; lack of precipitate suspension, lack of burns.
20	An increase in temperature was accompanied by a considerable increase in suspension thickness, which results in difficulties in mixing. Visible stagnated fragments of the mixed suspension. After gelatinization and termination of hydrolysis, visible layer of non-gelatinized oat meal at the bottom and on the walls of the flask, visible spots of suspension overheating (burns on the walls of flask), darker colour of the sample (as compared to the initial one). Burnt lumps containing non-hydrolyzed meal were found in the suspension.

Based on the results obtained and observations made, 15% concentration of comminuted oat kernels was adopted as a working concentration in further analyses.

Determination of the method of enzyme inactivation

In the procedure used for enzyme inactivation, Inglett [1990] applied overheated steam supplied under a pressure of 40 psi, which required specialist equipment. Laboratory trials conduced with the use of a steam generator without the possibility of pressure control fell short of expectations. The obtained pressure and temperature of steam were too low to inactive the enzyme in the entire sample volume in such a short period of time.

Taking this into account, in our study use was made of acidic inactivation [Bryjak, 1999]. The enzyme was inactivated with a solution of hydrochloric acid (2 mol/L) applied at a dose providing pH 3.5 of the suspension. The inactivation process spanned for 3 min.

Table 2 presents data of analyses conducted to determine the effect of the quantity of hydrochloric acid used for enzyme inactivation on DE values of the resultant hydrolysate. The first row of the table contains values of a glucose equivalent of the initial oat meal, whereas rows 2 and 4 – contain data on a glucose equivalent and final pH at various concentrations of hydrochloric acid used for the inactivation and at the same dose of the enzymatic preparation. In turn, rows 3 and 5 (zero samples) contain data obtained for samples without the addition of enzyme and with the same analytical procedure as the other samples. The hydrochloric acid was used in two doses, namely: 3.5 mL (samples 2 and 3) and 6.5 mL of 2 mol/L hydrochloric acid (samples 4 and 5).

The higher dose of the acid (6.5 mL) caused the final pH of samples 4 and 5 to oscillate between 1.8 and 1.9, whereas at its lower dose (samples 2 and 3), the final pH accounted for 3.3 and 3.2, respectively. As compared to sample 3, the increased dose of the acid (sample 5) evoked a considerable increase in DE value of the hydrolysate. In turn, the lower dose of the acid was observed not to affect DE values of the control samples (without the enzyme). A sample to which 6.5 mL of hydrochloric acid were added together with the enzyme was characterized by a twofold higher value of DE, elicited by more extensive acidic hydrolysis. The DE values assumed in the study were obtained in the case of samples supplemented with 0.5 mL of the enzyme inactivated with the lower dose of the acid (3.5 mL).

The application of hydrochloric acid was observed to inhibit an increase in DE value, but did not arrest the process of hydrolysis, which was due to further degradation of a starch molecule as a result of the initiated acidic hydrolysis. Arresting that process was possible only through neutralization of the acidified suspension with a 2 mol/L solution of sodium hydroxide until the final pH has reached 6.0. Simultaneously, assays were conducted to identify whether the applied procedures of enzyme inactivation and acid neutralization do not induce subsequent hydrolysis. To this end, an analysis of hydrolysate was carried out by collecting samples for 1 h in 10-min intervals and determining the DE value (Table 3).

The process of acid neutralization contributed to a single increase in DE value by *ca*. 0.1 unit on average. No further increase was observed in the content of monosaccharides within an hour since acid neutralization. Those analyses demonstrated that the time of enzyme inactivation should cover 3-min applications of 2 mol/L hydrochloric acid (pH=3.5), after which the sample should be neutralized with a 2 mol/L solution of sodium hydroxide (pH=6.0).

Determination of the susceptibility of oat meal to enzyme action

An aqueous suspension of oat meal (15%) was subjected to the action of an enzymatic preparation NERVANAZE for 3, 5, 7 and 10 min from the beginning of the hydrolysis process.

TABLE 2. Effect of the dose of hydrochloric acid used for enzyme inactivation on DE value of the resultant hydrolysate.

Sample	Enzyme dose (α-amylase, mL)	Dose of 2 mol/L HCl (mL)	Final pH	Glucose equivalent (LSD=0.1100)
Initial oat meal	-	-	-	0.3[a]
Oat meal subjected to complete procedure	0.5	3.5	3.3	3.6[c]
Oat meal subjected to the action of HCl used for enzyme inactivation	without enzyme	3.5	3.2	0.3[a]
Oat meal subjected to complete procedure	0.5	6.5	1.8	6.7[d]
Oat meal subjected to the action of HCl used for enzyme inactivation	without enzyme	6.5	1.9	1.5[b]

TABLE 3. Effectiveness of enzyme inactivation.

pH= 6.0	DE value and glucose content (%) in samples collected after specified time (in min) since neutralization at a temp. 70°C after:						
	neutralization	10'	20'	30'	40'	50'	60'
Glucose equivalent (LSD=0.1351)	4.5[a]	4.4[a]	4.5[a]	4.4[a]	4.5[a]	4.5[a]	4.5[a]
Glucose content (%) (LSD=0.0041)	0.073[a]	0.068[a]	0.070[a]	0.070[a]	0.072[a]	0.071[a]	0.074[a]

Constant parameters included: suspension concentration – 15% (75 g of meal mixed with 425 mL of water), enzyme dose – 0.5 mL, activity of the enzymatic preparation – 800-850 units acc. to SKB, pH of the reaction mixture – 6.0, temperature – 95°C; whereas a variable parameter was the time of enzyme action, *i.e.* 3, 5, 7, and 10 min. After the reaction of hydrolysis, the remaining quantity of enzyme was inactivated for 3 min with the use of 1 mol/L solution of hydrochloric acid. After inactivation, pH of the samples was adjusted to 6.0 using a 2 mol/L solution of sodium hydroxide. The value of the glucose equivalent that should reach 5.0±0.5 was adopted as a criterion of method optimization. Results were presented in Table 4.

TABLE 4. Susceptibility of oat meal to the activity of NERVANAZE-T preparation.

Enzyme action time (min)	Glucose equivalent (LSD=0.9561)	Glucose content (%)	β-Glucans content (%) (LSD=0.1935)
3	2.9[a]	0.051	4.6[b]
5	5.8[b]	0.127	5.0[a]
7	9.4[c]	0.150	5.1[a]
10	13.8[d]	0.222	5.3[c]

Experimental replications of the above-mentioned system enabled determining the time of enzyme application. A 3-min reaction allows for obtaining a hydrolysate with a glucose equivalent of 2.9. In turn, applying the enzyme for 5 min increases the DE value to 5.8, which corresponds to 0.127% of glucose. It is the target DE value of the elaborated hydrolysate. The longer reaction times (7 and 10 min) enabled obtaining the samples with substantially increased DE values. In the subsequent analyses, the time of 5 min was adopted as the optimal time of enzyme activity at its concentration applied in the study.

Table 5 as well as Figures 2 and 3 summarize results of the chromatographic separation of saccharides G1-G8 in the hydrolysate (Table 5, Figure 2) and OATRIM preparation (Table 5, Figure 3).

TABLE 5. Chromatographic analysis of G1-G8 oligosaccharides distribution in the hydrolysate and OATRIM preparation.

Saccharide	Retention time (min)		Peak area (uV . sec)		Peak area in respect of total oligosaccharides (%)	
	Hydrolysate	OATRIM	Hydrolysate	OATRIM	Hydrolysate	OATRIM
Fructose	20.933	20.850	42641	38314	1.24	1.14
Glucose	19.700	19.583	47971	41964	1.51	1.35
Maltose	18.717	18.717	-		-	
Maltotriose	17.250	17.150	83556	140159	3.62	4.52
Maltotetraose	15.383	15.317	113959	168715	4.58	5.44
Maltopentaose	13.850	13.800	88447	127711	3.78	4.12
Maltoheptaose	12.533	12.483	102474	147228	4.22	4.75
Maltooctaose	11.450	11.417	139895	187885	5.39	6.06
Higher saccharides	6.783	6.767	2244216	1909224	65.50	61.54
	9.317	9.300	87334	84517	2.74	2.72
	9.950	9.900	100357	107282	3.15	3.46
	10.633	10.583	132352	152439	4.16	4.91

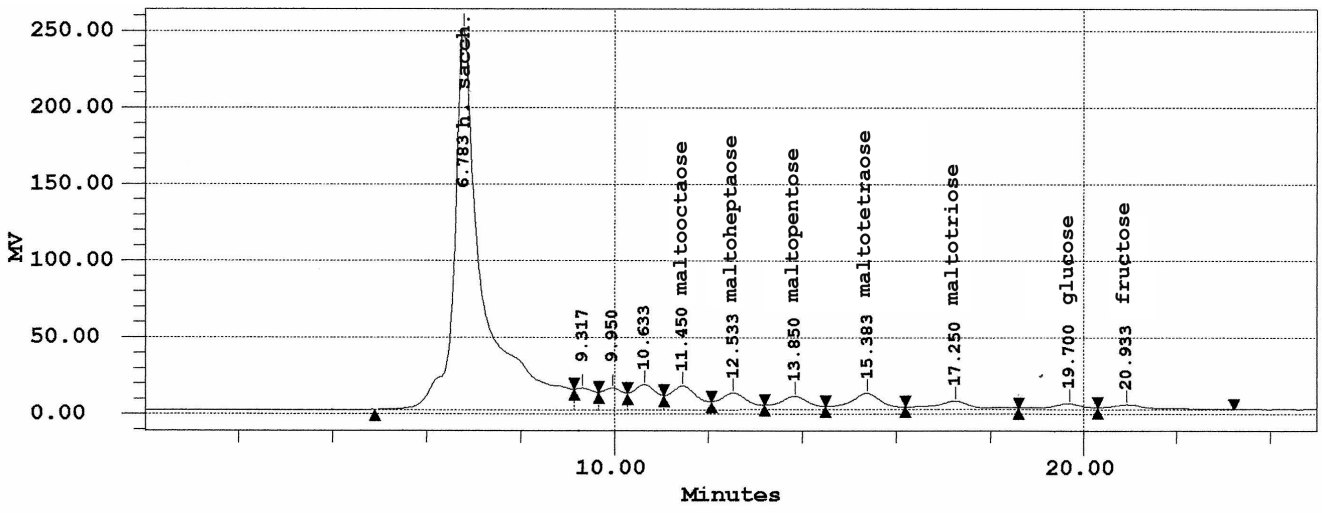

FIGURE 2. Chromatogram of HPLC separation of glucose oligomers G1-G8 in an oat hydrolysate.

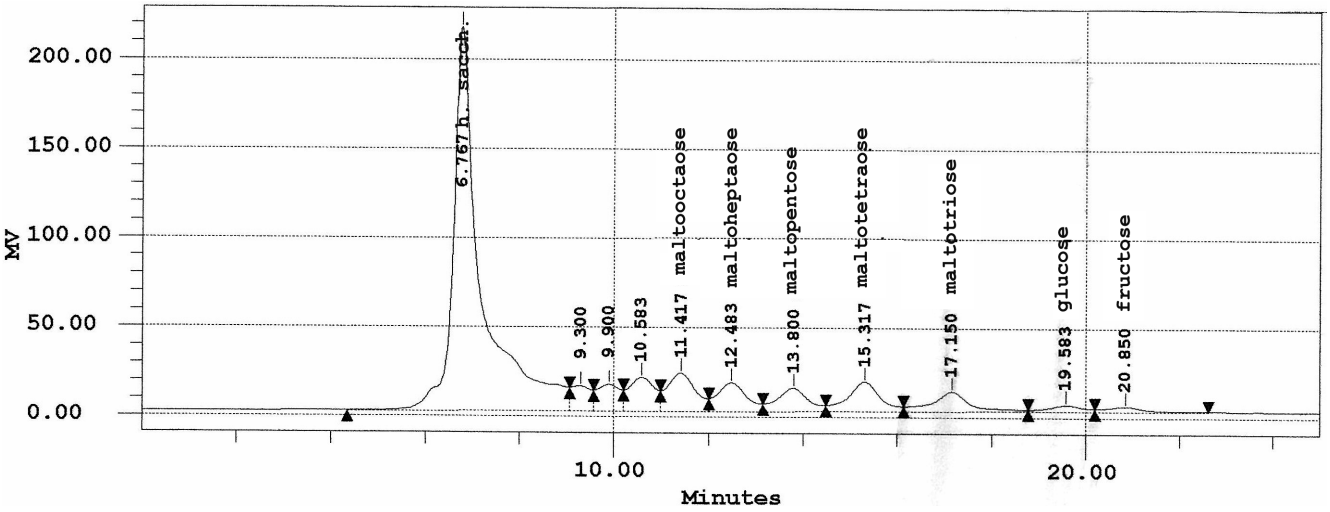

FIGURE 3. Chromatogram of HPLC separation of glucose oligomers G1-G8 in a commercial preparation OATRIM.

The presented chromatograms (Figures 2 and 3) do not demonstrate any significant differences between percentage contribution of oligosaccharides G1-G8 and are consistent with findings reported by Inglett [1990]. In the case of the hydrolysate, the total content of those oligosaccharides accounted for 24.34%, whereas in the OATRIM preparation – for 27.38%, yet the hydrolysate was characterised by a substantially higher content of monosaccharides: glucose and fructose, whereas the OATRIM preparation – by the higher content of oligosaccharides G3-G8.

CONCLUSIONS

1. The conducted study enabled improving the method of oat meat hydrolysis aimed at obtaining a low-saccharified hydrolysate with DE of 5.0±0.3.

2. The initial concentration of oat meal was specified as 15%, the time of the action of 0.5 mL dose of the enzyme – as 5 min, whereas acidic inactivation with 2 mol/L HCl should last for 3 min at pH 3.5 and be followed by acid neutralization with a sodium base (2 mol/L NaOH) to pH of *ca.* 6.0. The reaction of hydrolysis conducted under those conditions enables obtaining an oat hydrolysate with the glucose equivalent ranging from 4.7 to 5.2 and content of β-glucans reaching 5.0% on average.

3. The yield of the process from 10 subsequent samples of hydrolysis conducted under laboratory conditions ranged from 30% to 36% (23-27 g of hydrolysate on dry matter basis were obtained from 75 g of oat meal).

4. The percentage contribution of glucose oligomers G1-G8 in the oat hydrolysate is consistent with values of those parameters determined in a comparative preparation OATRIM.

REFERENCES

1. Anonymous, New Product News/GNPD Staff. A Strong Prelude. 2000, Prepared Foods. April, 67.
2. Anonymous, USDA's Oatrim replaces fat in many food products. Food Technol., 1990, 10, 100.
3. Brookes J.R., Griffin V.K., Saccharide analysis of corn syrup solids and maltodextrins using high performance liquid chromatography. Cereal Chem., 1987, 64, 253–262.
4. Bryjak J., Enzymatic hydrolysis of starch for maltodextrin and starch syrups. Part I. Enzymes. Biotechnologia, 1999, 44, 180–200 In Polish).
5. Dokic-Baucal L., Dokic P., Jakovljevic J., Influence of different maltodextrin on properties of O/W emulsion. Food Hydrocoll., 2004, 18, 233–239.
6. Fortuna T., Sobolewska J., Maltodextrins and their application in the food industry. Żywność, Nauka, Technologia, Jakość., 2000, 23, 100–109 (in Polish).
7. Gibiński M., Pałasiński M., Untesuchungen ueber die Staerke aus Polnischen hafersorten. Wissenschaftliche Zeitschrift der TH Koethen, 1991, 4, 45–52.
8. Gibiński M., Kowalski S., Sady M., Krawontka J., Tomasik P., Sikora M., Thickening of sweet and sour sauces with various polysaccharide combinations. J. Food Eng., 2006, 75, 407–414.
9. Hagberg S, A standardized Wohlgemuth methods for alpha amylase activity of wheat and rye. Cereal Chem., 1961, 58, 241–250.
10. ICC Standard Method no. 168 Mixed Linkage Beta-Glucan Assay Procedure.
11. Inglett G.E., Amylo-oligosaccharide formation from *B.stearothermophillus* α-amylase action on starches. J. Food Sci., 1990, 55, 560–561.
12. Inglett G.E., Newman R.K., Oat β-glucan-amylodextrins: Preliminary preparations and biological properties. Plant Foods Hum. Nutr., 1994, 45, 53–61.
13. Konuklar G., Inglett G.E., Warner K., Carriere C.J., Use of β-glucan hydrocolloidal suspension in the manufacture of low-fat Cheddar cheeses: textural properties by instrumental methods and sensory panels. Food Hydrocoll., 2004, 18, 535–545.
14. Łomnicki A., Wprowadzenie do statystyki dla przyrodników. 1999, Wydawnictwo Naukowe PWN, Warszawa, pp. 160–164 (in Polish).
15. Polish Standard, PN-EN-ISO-5377:2001. Products of starch hydrolysis. Determination of a reducing power and glucose equivalent (in Polish).
16. Richter M., Schierbaunm F., Augustat S., Knohch D., Method of producing starch hydrolysis products for use as food additives. 1976a, U.S. Patent 3, 962.465.

17. Richter M., Schierbaunm F., Augustat S., Knohch D., Process for the production of starch hydrolysis products. 1976b, U.S. Patent 1,423.780.
18. Roller S., Jones S. (ed), Handbook of Fat Replacers. 1996, CRC Press, Inc. New York.
19. Sandstedt R.M., Kneen E., Blish M.J., A standardized Wohlgemuth procedure for alpha-amylase activity. Cereal Chem., 1939, 16, 712.
20. Sampaio G.R., Castelluci C., Pinto e Silva M., Torres E., Effect of fat replacers on the nutritive value and acceptability of beef frankfurters. J. Food Comp. Anal., 2004, 17, 469–474.
21. Sawicka-Żukowska R., Zielińska K., Jędrychowska B., Enzymatic degradation of various types of native starch. Przem. Spoż., 1999, 5, 33–36 (in Polish).
22. Volikakis P., Biliaderis C.G., Vamvakas C., Zerfiridis G.K., Effects of a commercial oat β-glucan concentrate on the chemical and sensory attributes of a low-fat white-brined cheese product. Food Res. Int., 2004, 37, 83–94.
23. Walczycka M., Gibiński M., Migdał W., Achremowicz B., Tłuścik K., The quality of model sausages with addition of mash oat during 14 days of cold storage. Animal Sci., 2006, 1, 214–216.
24. Worrasinchai S., Suphantharika M., Pinjai S., Jamnong P., β-Glucan prepared from spent brewer's yeast as a fat replacer in mayonnaise. Food Hydrocoll., 2006, 20, 68–78.
25. Zgirski A., Gondko R., Obliczenia biochemiczne, 1998, Wyd. Naukowe PWN, Warszawa, 20–24 (in Polish).
26. Zoulias E.I., Oreopoulou V., Tzia C., Textural properties of low-fat cookies containing carbohydrate- or protein-based fat replacers. J. Food Eng., 2002, 55, 337–342.

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