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CHARACTERISTICS OF OLIGOSACCHARIDES PRODUCED BY ENZYMATIC HYDROLYSIS OF POTATO STARCH USING MIXTURE OF PULLULANASES AND ALPHA-AMYLASES

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ABSTRACT

Kinetics of amylolytic hydrolysis of autoclaved and extruded potato starch using alpha-amylase and pullulanase mixtures were described. Maltodextrins with dextrose equivalent of 3, 5, 8 and 12 were obtained and their sugar compositions with HPLC method were analyzed. The low molecular sugar (PD 1-2) and oligosaccharide (PD 3-8) contents as well as relationship between dextrose equivalent of maltodextrins and oligosaccharide contents were determined.

Key words: maltodextrin, oligosaccharide, amylase, pullulanase, hydrolysis.

INTRODUCTION

Starch consists of two glucose polymer carbohydrates: amylose and amylopectin. Amylose is an essentially linear, long chain polymer (α-1,4-D-glucan) with a molecular weight of 10^5-10^7. Amylopectin is a branched carbohydrate with a molecular weight of 10^5-10^8. One branch by α-1,6-D-linkage falls to about 20-25 α-1,4-D-bound glucose units. The contribution of the individual fractions in starch structure depends on the botanic source and conditions of plant cultivation. In potato tubers the amylose content amounts to about 20%.

A fast and efficient hydrolysis requires starch pre-swelling in water and full starch gelatinization. In practice, it can be achieved by incubation of moistened starch at a temperature exceeding 100°C, although, the minimal reported gelatinization temperature for potato starch is 59°C. During the gelatinization process, swelling of starch granules, loss of their crystalline structure and increase of solubility are observed. Usually, starch gelatinization is carried out by autoclaving in a high pressure cooker or by extrusion.

The enzymatic starch hydrolysis with alpha-amylases produces a mixture of branched α-dextrins, short linear oligosaccharides, maltose and glucose [8, 12, 16]. Branched dextrins can be further cleaved to short linear oligosaccharides by isoamylases and pullulanases. The degree of starch hydrolysis is usually expressed as the dextrose equivalent (DE), which is measured as the total reducing power of all sugars present in the reaction of the reduction of Cu^{++} to Cu^{+} in Fehling's solution, relative to glucose as 100 and expressed on a dry weight basis.

Food industry is mainly interested in starch hydrolysates with a limited number of cleavages of glycosidic bond inside the starch macromolecules and a low contents of glucose and maltose. Especially attractive are maltodextrins with a DE below 20 because of their functional properties, such as ability to gel and paste, the stabilization of water/oil emulsions, the inhibition of large size ice crystal formation, the use of maltodextrins for encapsulation, the formation of carbohydrate films, to name just a few [3].

The technological properties of maltodextrins are determined first of all by their chemical composition. There are many reports of hydrolysis kinetics related to the solution of low starch concentration [1, 4, 5, 7, 10, 17, 18] . The reduction potential of starch hydrolysates expressed as the dextrose equivalent is usually presented as a function of reaction time. This type of information is of limited importance because the hydrolysates with the same dextrose equivalent can significantly differ in their carbohydrate composition. An important characteristic of maltodextrins is their oligosaccharide composition. The knowledge of the hydrolysis course makes easier the choice of the enzyme preparation and reaction conditions. Marchal et al. [13], in a review paper, stressed the necessity of fundamental research on the chemical composition of oligosaccharides produced in the reaction of starch hydrolysis. A good knowledge of the chemical composition of hydrolysates makes it possible to predict their technological, nutritive and rheological properties. For example, it is well known that maltotriose (DP3) shows the highest hygroscopicity, whereas maltose (DP2) the lowest [6]. The chemical composition of maltodextrins also influences the viscosity of their aqueous solutions [9]. For the oligosaccharides with DP 1-7, the viscosity of solutions increases linearly with the increase of the molecular weight and sugar concentration. With a higher polymerization degree, these relationships are curvilinear. The length of the oligosaccharide chains also affects the sweetness of their solutions. Above DP 7 the sweetness of oligosaccharides is not perceptible. Other relations between polymerization degree and functional properties of oligosaccharides could also be mentioned.

The aim of this work is to determine the relationship between the enzymatic preparation used for starch hydrolysis and the chemical composition of maltodextrins obtained in the process whose dextrose equivalent was equal to 3-12.

MATERIALS AND METHODS

Starch

Potato starch Superior used in this study was purchased from Zakłady Przemysłu Ziemniaczanego (Lubon, Poland). Before enzymatic hydrolysis starch was gelatinized by two processes: the autoclaving and the extrusion.

Gelatinization by autoclaving

A 5% w/v starch suspension in deionized water was heated to 70°C. Then, an additional portion of native starch was added and kept at this temperature for 15 min at slow stirring in order to attain a completely suspended matter of 10% w/v starch concentration. The suspension was placed in a closed vessel ventilated through an air filter and autoclaved at 121°C for 20 min. Afterwards, the solution was cooled to the temperature of enzyme reaction to be used for further study.

Gelatinization by extrusion

Potato starch was moistened to 35% moisture content with deionized water and mixed in a laboratory helical agitator at 20°C to unify the water content. The process of starch extrusion was carried out in a twin-screw extruder model ZSK 25P8.2 (Krupp Werner & Pfleiderer, Germany) at a temperature of 105°C and a continuous stirring rate of 80 rpm/min. The product came out from the extruder head in the form of noodles which were then cut into pieces 1-1.5 cm long. The extrudate was dried at an ambient temperature for two days. Afterwards, the dried pieces were milled in an impact mill into powder form.

Enzyme preparations
Starch hydrolysis was carried out with the following enzyme preparation purchased from Novo Nordisk (Denmark): BAN 480L (bacterial α -amylase; 65°C, pH 6.5), Fungamyl 800L (fungal α -amylase; 60°C, pH 5.0), Promozyme D and Promozyme 200L (fungal pullulanase; 60°C, pH 5.0).

Enzymatic hydrolysis
Enzymatic hydrolysis was conducted using 10% w/v gelatinized starch concentration at a temperature of 60°C and pH 6.5 for 60 min. The doses of enzyme preparations were used as follows (activity units per 1 kg starch):

- BAN480L 1 KNU + Promozyme D 0.6 NPUN,
- BAN480L 1 KNU + Promozyme 200L 2.5 KNU,
- Fungamyl 800L 0.4 KNU + Promozyme D 0.6 NPUN,
- Fungamyl 800L 0.4 KNU + Promozyme 200L 2.5 KNU.

The reaction was carried out in a shaking water bath in closed glass vessels. The reaction was stopped by lowering pH to 3.5 with citric acid followed by boiling for 5 min. The samples were collected during the hydrolysis phase at 10 min intervals.

Dry matter
Dry matter content in starch was determined gravimetrically in triplicate in a drying oven at 105°C until constant weight was achieved.

Dextrose equivalent
The values of dextrose equivalent were determined using titration method based on the reduction of Cu^{2+} to copper(I)-oxide according to Lane and Eynon [11].

Determination of sugars by HPLC
Oligosaccharide composition ranging from glucose (DP1) to maltooctaose (DP8) was determined by the HPLC method using a Hewlett-Packard Model 1050 chromatograph, equipped with an Aminex HPX-42A column (300 mm x 7.8 mm; Biorad), a pre-column and an index refraction HP1049 detector. The samples were eluted with deionized water at a flow rate of 0.6 ml/min operated at temperature 85°C. The samples were previously diluted to 5°Bx, passed through a 0.22 µm filter and pre-column, and loaded onto a column at a volume of 25 µL. The individual sugars were identified on the basis of retention time. The quantitative determination of sugars was done using the measurement of height of peaks and computer integration.

RESULTS AND DISCUSSION

Kinetic studies
The enzymatic hydrolysis of potato starch was performed using different enzyme preparations and on this basis the reaction kinetics were determined as a function of dextrose equivalent and reaction time. The dextrose equivalent was used as a measure relating the hydrolysis degree to reducing sugar production. The kinetics were expressed in the form of regression equations valid for 60 min of enzymatic reactions. The aim of this study was to define the reaction time required to produce maltodextrins with desired DE values equal to 3-12. This is the main group of maltodextrins desired by the food industry for their functional properties. The results of this study are presented in Table 1.

Table 1. Regression equations describing the course of starch hydrolysis performed with different enzyme preparations

Enzyme Preparation	Hydrolysis time min						Regression equations (reaction time: 60 min)
	10	20	30	40	50	60	
	DE values of hydrolysates obtained by hydrolysis of autoclaved starch						
Promozyme D + BAN	4	7	10	14	17	21	Y=0.3314x + 0.6667
Promozyme D + Fungamyl	4	7	10	12	15	17	Y=0.256x + 1.9067
Promozyme 200L + BAN	3	7	11	13	15	17	Y=0.2611x + 2.0267
Promozyme 200L + Fungamyl	3	5	8	10	11	13	Y=0.2006x + 1.2467
	DE values of hydrolysates obtained by hydrolysis of extruded starch						
Promozyme D +BAN	3	7	9	13	17	19	Y=0.3231x + 0.1067
Promozyme D + Fungamyl	2.5	6	9	11	13	16	Y=0.2606x + 0.6133
Promozyme 200L + BAN	3	6.5	8	13	16	17	Y=0.2829x + 0.9333
Promozyme 200L + Fungamyl	3.5	6	8	12	13	16	Y=0.2583x + 1.06

It was found that the relationships between the DE values versus reaction time were linear. The data obtained show that the hydrolysis rate using the starch gelatinized by autoclaving was higher than the starch gelatinized by extrusion but the differences can be assumed to be low. However, it was noted that the differences between the DE values caused by the use of different enzyme cocktails increased in time. The use of the autoclaved starch resulted in more differentiated products than the use of the extruded starch.

In general, the enzyme cocktails containing the BAN preparation were more efficient that those containing Fungamyl. The highest reaction rate was obtained in the hydrolysis of autoclaved starch with an enzyme mixture of Promozyme+BAN where the DE reached the value of 21 after 60 min. This was only the case when the final DE value exceeded 20.

On the basis of kinetics data, it was possible to define precisely the reaction time needed to obtain the maltodextrins with desirable DE values: 3, 5, 8, and 12. These products were used in further study for the analysis of oligosaccharide composition.

Oligosaccharide profiles of maltodextrins

The maltodextrins produced in hydrolysis reactions consist of a mixture of high polymerized saccharides, oligosaccharides and glucose. As was expected, the use of different enzyme cocktails resulted in different hydrolysis products, thus, a range of maltodextrins with unique oligosaccharide profiles were obtained.

Data presented in Figures 1 and 2 indicate that the preparation containing α-amylase had the main effect on the differentiation of sugar composition. According to manufacturer's information, the BAN 480L preparation contains bacterial α-amylase produced by *Bacillus licheniformis*, whereas the Fungamyl 800L preparation contains fungal α-amylase produced by *Aspergillus niger*.

In the present investigation, the oligosaccharides with a polymerization degree of 3 (maltotriose) to 8 (maltooctaose) were considered. The data presented in Figures 1 and 2 show significant differences in concentration of individual oligosaccharides in hydrolysates and their pattern while the DE values increase. Independently of the gelatinization mode, the concentrations of oligosaccharides in the hydrolysates with the DE values of 3 to 8, obtained by the use of the mixture of Promozyme + Fungamyl preparations, were very similar and comprised a narrow range of values. Distinct differences in concentration of individual sugars appeared only in maltodextrins with DE 12.

Fig. 1. Oligosaccharide concentration in maltodextrins produced from autoclaved starch and various enzyme preparations as a function of DE (hydrolysis conditions: starch concentration 10 % w/v, temp. 60°C, pH 5.0)

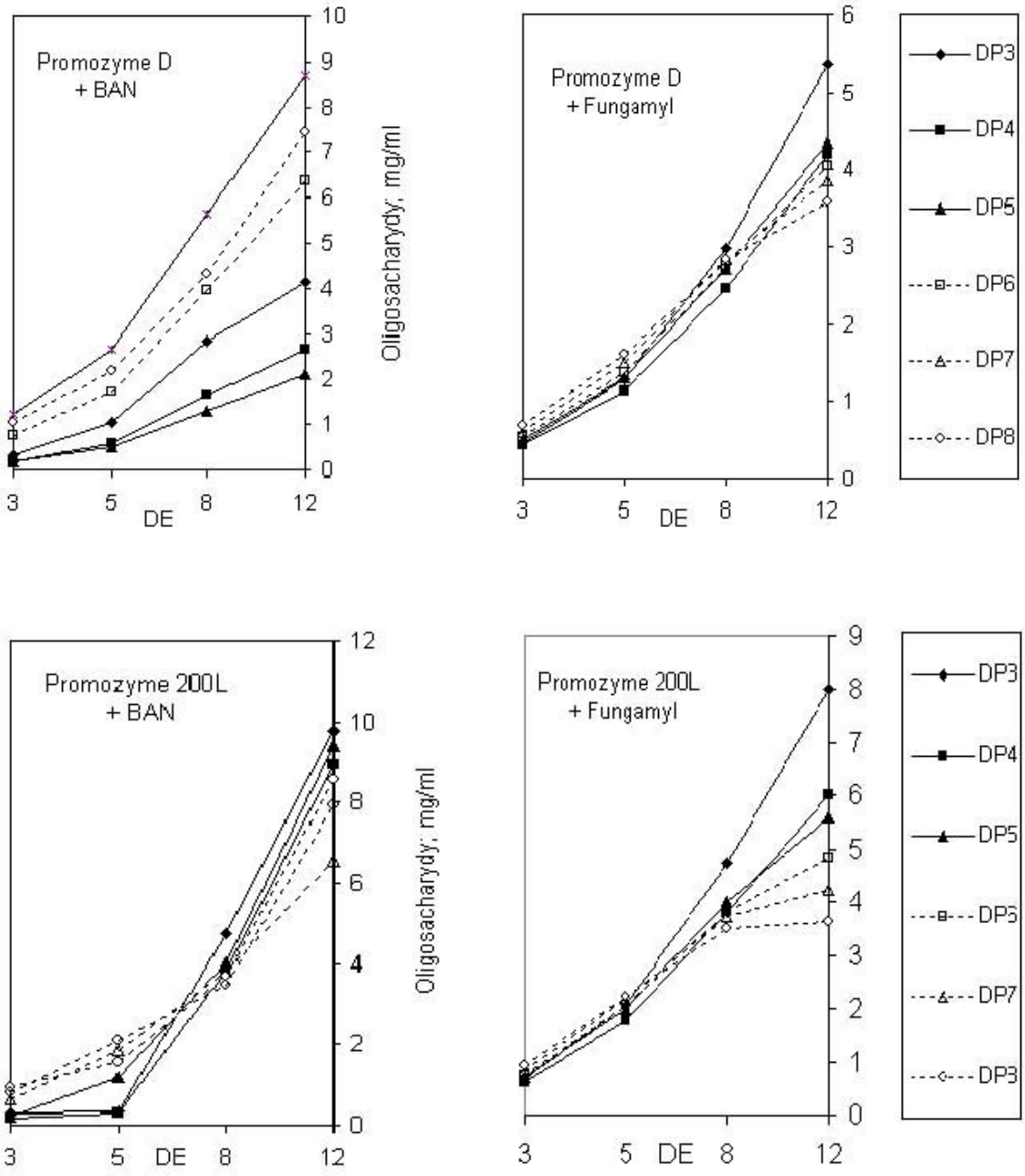
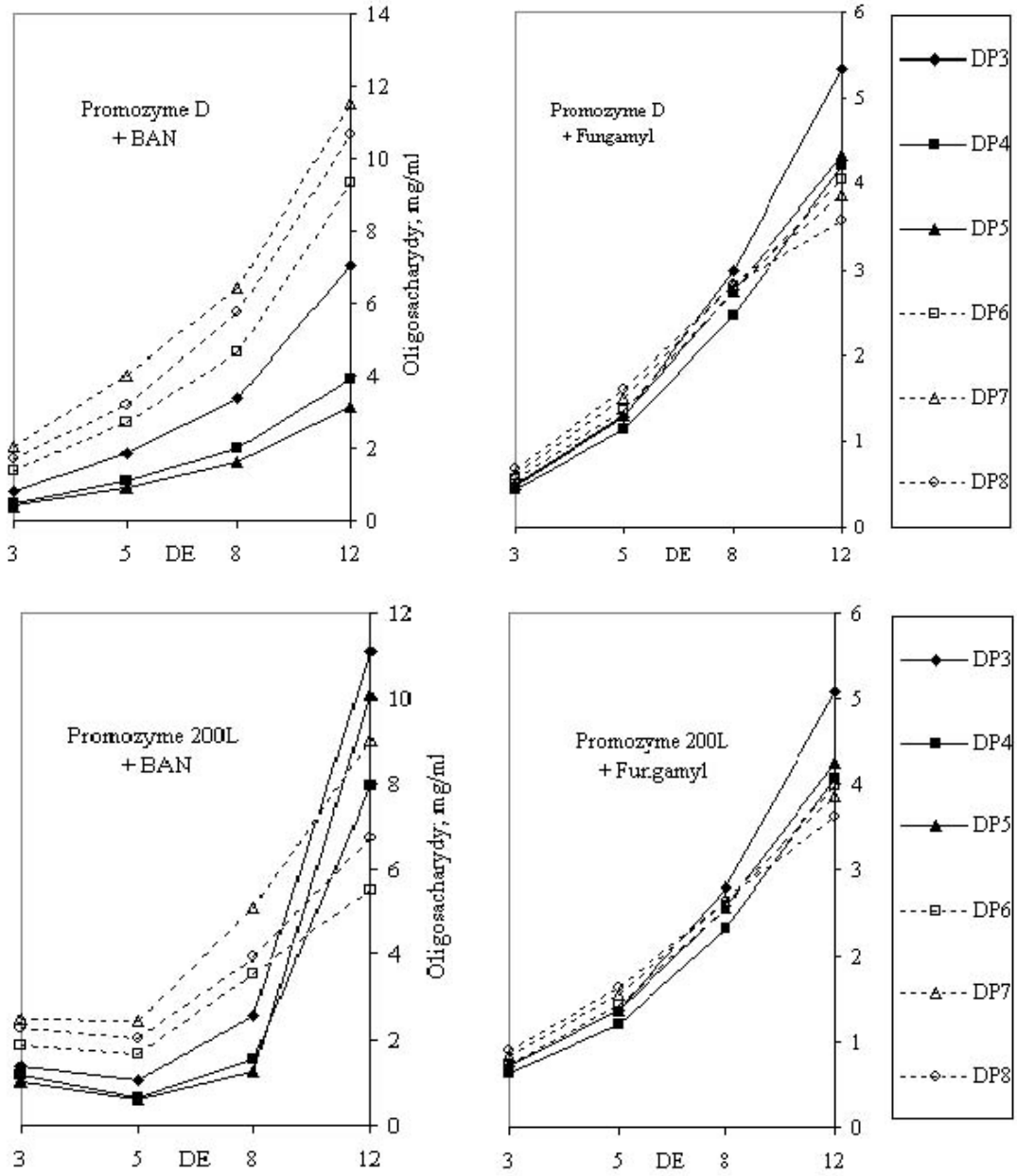


Fig. 2. Oligosaccharide concentration in maltodextrins produced from extruded starch and various enzyme preparations as a function of DE (hydrolysis conditions: starch concentration 10 % w/v, temp. 60°C, pH 5.0)



When the autoclaved starch was used, the concentrations of individual oligosaccharides varied in the range of 3.5 mg/ml (DP8) – 5.3 mg/ml (DP3) for Fungamyl + Promozyme D mixture, and 3.6 mg/ml (DP8) – 8.0 (DP3) for Fungamyl + Promozyme 200L mixture (Fig. 1). Interestingly, in these maltodextrins the highest concentration was obtained for the oligosaccharides with a low polymerization degree ranging from 3 to 5, where a significantly high concentration of maltotriose reaching over 5 mg/ml was observed.

For the same starch substrate, when BAN preparation was present in the enzyme mixtures, the concentration of individual sugars in low DE maltodextrins was more differentiated. High molecular weight sugars appeared in a higher concentration than low DP sugars. Also the range of individual oligosaccharide concentrations in maltodextrins with DE 12 was larger. This varied from 2.1 mg/ml (DP5) to 8.7 mg/ml (DP8) for BAN + Promozyme D mixture, and from 2.3 mg/ml (DP5) to 8.5 mg/ml (DP8) for BAN + Promozyme 200L mixture.

Many interesting observations were made after the chromatographic analysis of sugars in maltodextrins produced with Promozyme + BAN preparations. First, a higher differentiation in concentration of individual sugars in the whole DE range was demonstrated in comparison to maltodextrins produced with Promozyme + Fungamyl preparations. The differentiation of individual sugar concentration increased with the increase of DE

value in a solution of starch hydrolysates. A different trend was also observed in the relations between the oligosaccharides with a low polymerization degree and a high polymerization degree (DP 6-8).

When the enzyme cocktails composed of Promozyme + BAN were used, the concentration of oligosaccharides with DP 6-8 was distinctly higher. This tendency was observed independently of the mode of starch gelatinization before hydrolysis and the type of pullulanase preparation used in the enzyme cocktails (Promozyme D or Promozyme 200L).

An interesting observation was made when the composition of maltodextrins with DE 8 was compared to the composition of the commercial product Paselli MD10, produced by AVEBE America Inc. According to Wang and Wang [19], the individual sugar content in this maltodextrin is as follows [% d.m.]: glucose 0.64, maltose 1.47, maltotriose 2.84, maltotetraose 1.57, maltopentaose 2.45, maltohexaose 3.26 and higher sugars 87.77. This means that the maltodextrins obtained in this investigation are different in sugar composition. First of all, the content of low molecular weight sugars (DP 1-2) was distinctly higher in the commercial product than in maltodextrins obtained in this work (Table 2). Distinct differences were observed when comparing the content of the sugars with a higher degree of polymerization. In general, the commercial maltodextrin contains less oligosaccharides with longer chains (DP range from 3 to 7). There are also differences in hydrolysates made from autoclaved and extruded starch. It can be expected that the differences in chemical composition will also result in differences in functional properties of maltodextrins.

Table 2. Content of low molecular sugars and higher sugars in maltodextrins produced using mixture of alpha-amylases and pullulanases

Enzymes	DE	Autoclaved starch			Extruded starch		
		Sugar contents, %			Sugar contents, %		
		Glucose	Maltose	Higher sugars	Glucose	Maltose	Higher sugars
PromozymeD + BAN	3	0.03	0.09	96.17	0.10	0.26	92.95
	5	0.09	0.34	90.83	0.14	0.63	85.58
	8	0.16	1.01	75.18	0.22	1.23	74.83
	12	0.54	1.67	66.38	0.44	2.86	51.05
PromozymeD + Fungamyl	3	0.06	0.13	96.60	0.06	0.13	96.60
	5	0.06	0.34	91.40	0.06	0.34	91.41
	8	0.07	0.80	82.57	0.07	0.80	82.56
	12	0.09	1.60	72.93	0.09	1.60	72.93
Promozyme 200L + BAN	3	0.10	0.16	96.73	0.10	0.36	89.42
	5	0.12	0.66	91.90	0.13	0.39	91.09
	8	0.14	1.43	74.83	0.18	0.94	81.02
	12	0.34	1.99	48,85	0.32	1.84	47.42
Promozyme 200L + Fungamyl	3	0.07	0.20	95.30	0.07	0.23	95.16
	5	0.13	0.60	86.92	0.11	0.41	90.92
	8	0.14	1.43	74.83	0.11	0.79	83.50
	12	0.14	2.72	64.87	0.15	1.54	73.47

Marchal et al. [14] reported that in the hydrolysates of potato starch obtained by the use of α-amylase from *Bacillus licheniformis* (Maxamyl, Gist-Brocades, presently Genencor, Netherlands) a strong correlation between DE and glucose and maltotetraose concentration was noted, although, the relation of maltopentaose concentration to DE values was rather unstable. This was not observed in our investigation. Marchal et al. [15] carried out an extensive study on the effect of temperature on the saccharide composition of starch hydrolysates in the DE range from 1-30. The data presented by these authors showed that the glucose contribution in the total sugar composition increased slowly while the DE value increased up to DE 20 and then the glucose content increased very rapidly up to DE 30. The maltose and maltotriose content in the hydrolysates changed continuously with the DE increase from 1 to 30. The relationship between maltohexaose and maltoheptaose contribution in total carbohydrates exhibited a different pattern. The maximum content of maltohexaose was noted at DE 18-25 depending on the hydrolysis temperature, whereas the highest maltoheptaose concentration

appeared at DE 14-20 and then rapidly dropped to about DE 25, followed by a second increase. In the DE range below 10, the oligosaccharide content in maltodextrins increased linearly which confirmed our observations. A comparative study on maltodextrin structures from different biological sources was published by Wang and Wang [19], who analyzed the molecular distribution of carbohydrates in corn, rice and potato maltodextrins as well as the profiles of these maltodextrins after enzymatic treatment with debranching isoamylase from bacteria *Pseudomonas* sp. The behaviour of this enzyme is similar to the pullulanase used in this work. The HPLC profile of debranched potato maltodextrin differs from the native maltodextrin with higher content of maltotriose and sugars with DP 10-30.

CONLUSIONS

1. Kinetics of starch hydrolysis showed that hydrolysis rate of extruded starch is lower than that of autoclaved starch.
2. Maltodextrins with the same DE value differs in the sugar composition which is the result of the use of different mixtures of enzyme preparations and different modes of starch gelatinization.
3. Maltodextrins obtained with the use of Promozyme+BAN mixture are characterized by a higher differentiation in individual oligosaccharides than the maltodextrins obtained with the use of Promozyme+Fungamyl preparations.
4. The use of Promozyme + BAN preparations results in the production of larger amounts of higher polymerized oligosaccharides (DP 6-8).
5. Maltodextrins with DE 8-12 obtained in this work significantly differ from commercial maltodextrin Paselli MD10 AVEBE.

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