ACTA SCIENTIARUM POLONORUM Technologia Alimentaria 2(2) 2003, 17-26

LIQUEFACTION OF STARCH BY THERMOSTABLE ALPHA-AMYLASE*

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Abstract. Starch hydrolyzates were prepared by treatment of starch (potato, corn, tapioca) with heat-stable alpha-amylase [EC 3.2.1.1.] and the dextrose equivalent, carbohydrate composition, viscosity and filtration rates were measured in obtained hydrolyzates. The differences of susceptibility of the tested starches to Termamyl 120 type LS action are small (less than 2 DE). Hydrolyzates with similar DE characterise the similar carbohydrate composition in spite of the use of a different kind of starch as a substrate. Filterability and viscosity of hydrolyzates depends on the kind of starch. Mixtures of tapioca and corn starches were shown to give hydrolyzates with similar viscosity to potato starch hydrolyzates.

Key words: starch, alpha--amylase, hydrolyzates, carbohydrate, filtration rate, viscosity

INTRODUCTION

Starch molecules are composed of essentially linear polymer of glucopyranose units consisting predominantly of α-D-(1$\rightarrow$4)-linkages and branched amylopectin containing chains with a short degree of polymerization (DP = 20 to 25 glucopyranose residue) linked to the C-6 hydroxymethyl position of certain glucose moieties via α-D-(1$\rightarrow$6)-linkages [Biliaderis 1991, Greenwood and Starch 1976]. Technological properties of starch depend upon the botanical sources from which it is isolated and are related to the shape and size of starch granules, as well as the proportion in quantity of amylose to amylopectin, the content of fat, proteins and non-starch polysaccharides. Corn and tapioca starches differ from potato starch in their lipid and protein content, granule size, colour and viscosity. Corn grain starch contains considerably more fat (corn starch 0.87%) than tapioca starch (0.1%) or potato starch (0.05%) [Słomińska 1997, Swinkels 1985].

The purpose of this work was to compare the influence of the thermophilic enzymatic preparation action – Termamyl 120 Typ LS – on different sources of starch.

*This study has been carried out with financial support from the State Committee of Scientific Research (grant PBŻ-KBN 021/P06/13/2001)

MATERIAL AND METHODS

Starch

Potato starch (Factory of Potato Industry – Poland), corn starch (Avebe – Holland) and tapioca starch (Berkeamidon – Thailand) were used.

Enzyme

Termamyl 120 type LS (Novo Nordisk, Denmark) – a mixture of outstanding heat-stable alpha-amylases produced by selected strains of *Bacillus licheniformis.* The enzyme activity was 120 KNU/g (KNU = Kilo Novo Units alpha-amylase – the amount of enzyme which breaks down 5.26 g of starch per hour at Novo's standard method for determination of alpha amylase). According to Novo, Termamyl LS in comparison with Termamyl L has a higher heat stability and lower pH tolerance (5.0-5.9).

Analysis

The following determinations were carried out: the content of reducing sugars according to the modified Schoorl-Rogenbogen method [Rauscher 1956], the carbohydrate composition by HPLC using Ostion LG/KS 0803 column (samples – 20μL – were eluted at 1.5 $cm^3 \cdot min^{-1}$ using water at 85°C), the viscosity measured in Brabender Typ E and at 50°C using Rheotest RV 2, the filtration rate (the volume of hydrolyzate – ml – filtered through Büchner filter of a diameter 7 cm covered filter paper under pressure 60 kPa within 1 min), the colour factor using Minolta CR-300 series Chroma Meters, the ash content by Kieldhal's method [Pearson 1976], the total and soluble protein content by the Lowry's method [Lowry et al. 1972], the lipid content [PN-EN ISO 2001], the average diameter of starch performed in Analysette 20 f-ma Fritsch.

Procedure

Starch was slurried in water to obtain a starch concentration of 35% d.s. The pH-value was adjusted to 5.9 by 20% citric acid and enzyme dosage of Termamyl LS in amounts of 60; 85; 125; 145 NU/g DS was added**.** Ca^{++} in amount of 30 ppm was used as an activator. The suspension was heated to 95°C and maintained for 3 h. The enzyme was inactivated by citric acid. The reaction mixtures were sampled at 1 h intervals. DE and carbohydrate composition in each sample was measured.

RESULTS AND DISCUSSION

Starch properties

The properties of the tested starches used as substrates for alpha-amylase action are shown in Table 1. The results confirm differences that exist between starches depending on the sources from which they are isolated.

Table 1. Properties of tested starches
Tabela 1. Właściwości badanych skrobi

Properties – Właściwości	Starch – Skrobia		
	potato ziemniaczana	corn kukurydziana	tapioca tapiokowa
Colour – Barwa	L* = 93.66□a** = –1.53□b** = +1.34	L = 94.68□a = –2.78□b = +5.70	L = 95.21□a = –1.35□b = +1.67
Humidity, % – Wilgotność, %	19.5	10.0	12.0
pH	6.2	5.7	5.2
Content of ash in d.s., % Zawartość popiołu w s.s., %	0.26	0.14	0.20
Content of protein in d.s., % Zawartość białka w s.s., %	0.05	0.36	0.10
Content of lipid in d.s., % Zawartość tłuszczu w s.s., %	0.03	0.20	0.08
Avarage diameter of starch granule, μm Średnia średnica ziarenek skrobi, μm	36.0	14.0	14.2

*L – lightness factor.
**a, b – chromatically coordinates.
*L – jaskrawość.
**a, b – współrzędne chromatyczne.

The measurement of absolute starch colour by the L*a*b* colour system indicates that potato starch has the best colour parameters. Negative value of chromaticity coordinate "a" which indicate affinity to green colour and positive value of chromaticity coordinate "b" which indicate affinity to yellow colour are the lowest for potato starch.

The corn starch has the highest amount of protein and fat. It is well known that the content of protein in hydrolyzates production is undesirable because it is responsible for colour and odour creation, as well as foam forming in the process of starch hydrolysis [Bowler and Towersey 1985]. A high percentage of lipids in starch used as a source of substrate for hydrolyzates production is also disadvantageous. The negative effect is observed of amylose-lipids complexes on the ability of water assimilation and swelling. They also cause opalescent and cloudiness of starch slurry [Konieczny-Janda and Richter 1991] and worse filtration characteristic [Matser and Steeneken 1998].

The size of tested starches granules is different. The potato starch granules diameter is almost three times higher than tapioca and corn starches.

Enzyme dosage

The influence of enzyme dosage on starch hydrolysis depends on the kind of starch. After 1 h of enzyme action in dosage 60-120 NU/g d.s. on corn starch the value of DE varies from 8.9 to 18.2, on potato starch from 8.5 to 17.9 and on tapioca from 7 to 16.8. As shown in Figure 1 A, the differences in DE for the same enzyme dosage are small, less than 2 units. Moreover, the highest enzymatic susceptibility of corn starch and the lowest tapioca starch can be observed.

Effect of kind of starch on the increase of dextrose equivalent during 3 h of hydrolysis is also illustrated by Figure 1 B. The differences in DE fluctuate from 0.5 to 4. Applying of enzyme dosage in amount of 120 NU/g d.s. gives the highest DE for corn starch. DE amounts as follows: after 1 h – 17.1, after 2 h – 23.1 and 3 h – 24.1.

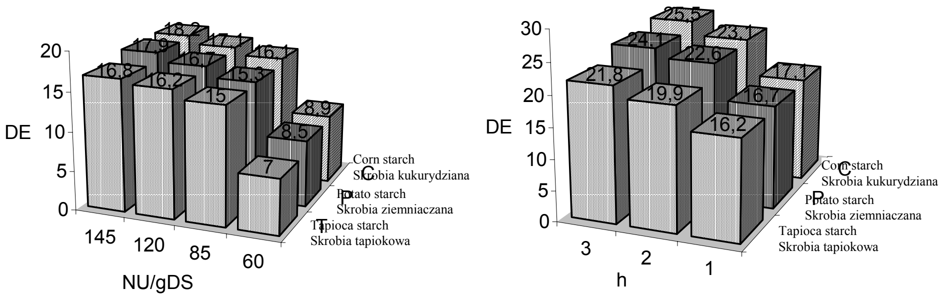

Fig. 1. Influence of the starch source on the polymerization degree: A – dependence on the enzyme dosage, B – dependence on hydrolysis time. Liquefaction conditions: 35% starch, pH – 5.9, temp. – 95°C, A – time 1 h, B – enzyme dosage 145 NU/g d.s.
Rys. 1. Wpływ źródła skrobi na stopień polimeryzacji: A – wpływ dawki enzymu, B – wpływ czasu hydrolizy. Warunki upłynniania: 35-procentowa zawiesina skrobi, pH 5,9, temp. 95°C, A – czas 1 h, B – dawka enzymu 145 NU/g s.s.

These results agree with those found by Niemann at al. [1995]. They ascertained that degree of degradation of starches after hydrolysis with thermostable alpha-amylase preparation Thermamyl 120L amounts for corn starch 22,4% but for tapioca starch 20,9%. Franco et al. [1987] proved that starches which naturally show a porous surface, as in the corn starch, were degraded easier than with a smooth surface as in tapioca starch. Bertoft and Kulp [1986], MacGregor and Ballance [1980], Franco et al. [1988] found that the percentage of hydrolysis increase with decrease of granule size.

Carbohydrate composition

The starch hydrolyzates characterised by approximate DE (25.3-26.5) value have similar starch composition (Fig. 2). For example hydrolyzates with DE within the range 25-27 have glucose content as follows: corn hydrolyzates 1.8%, tapioca hydrolyzates 1.8% and potato hydrolyzates 2.0% and maltose – 14.2, 12.8, 14.6, respectively.

The results suggest that *Bacillus licheniformis* amylase produce mainly two oligosaccharides G_3 and G_6. Similar action effect was observed also by the use of alpha amylase from *Bacillus subtilis* on hydrolysis of corn starch [Brooks and Griffin 1987, Lovšin et al. 1998, Scobell and Brost 1981].

Amount of G_n in the hydrolyzates is similar for tested starches according to their similar DE. Literature data indicate that low DE corresponds to higher MW saccharides. Wang and Wang [2000] showed that potato maltodextrin with 5.9 DE was composed of a higher percentage of saccharides with DP ≥ 10 than corn maltodextrin with 8.2 DE. However, Griffin and Brooks [1989], in spite of similar DE of maltodextrin obtained by Termamyl 120L treatment of milled rice flour, obtained different proportions of high and low molecular weight saccharides.

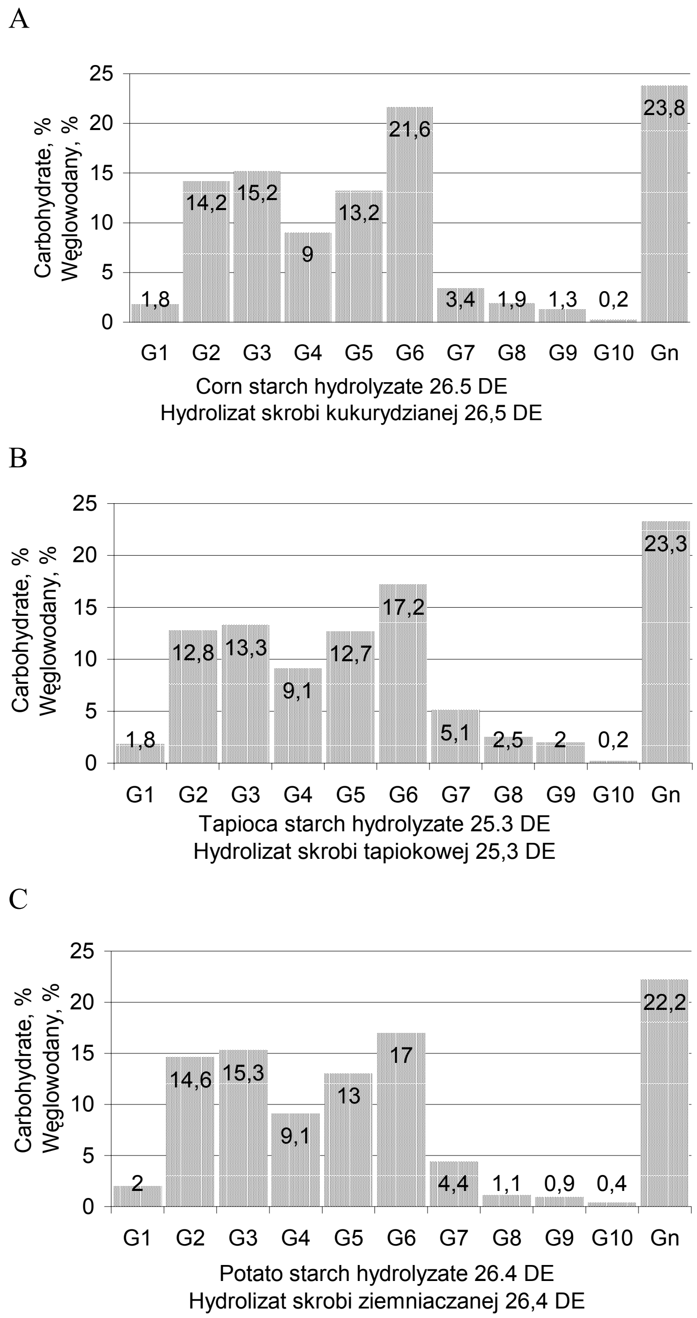

Fig. 2. Gel chromatograms of the carbohydrate spectra of the enzyme liquefied starches, G_1-G_n – oligomers containing up to n glucose residue
Rys. 2. Skład węglowodanowy enzymatycznie upłynnionej skrobi, G_1-G_n – oligomery zawierające reszty glukozowe

Filtration rate

Filtration rate of hydrolyzates obtained after 3 h of enzyme action in amount of 145 NU/g d.s. depends also on the type of starch. The highest filtration rate indicates tapioca hydrolyzates – 15 ml/min. It is two times higher than potato hydrolyzates and three times higher than corn starch (Fig. 3). It can be observed that filtration rate of starch hydrolyzates varies significantly in spite of their similar dextrose equivalent.

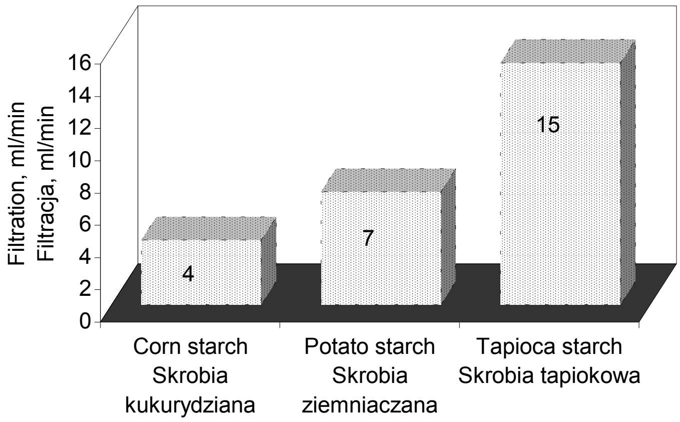

Fig. 3. Filtration rate of the enzymatic liquefied starches. Liquefaction conditions: 35% d.s. starch, time – 3 h, enzyme dosage – 145 NU/g d.s., pH – 5.9
Rys. 3. Szybkość filtracji skrobi upłynnionej enzymatycznie. Warunki upłynniania: 35-procentowa zawiesina skrobi, czas – 3 h, dawka enzymu – 145 NU/g s.s., pH – 5,9

Viscosity

The comparison of tested starch viscosity is illustrated in Figure 4. The Brabender measurement was made for starches without enzyme and with enzyme addition. Maximum viscosity without enzyme addition is as follows: potato starch equal 2700 BU (Brabender units) at 75.5°C, tapioca starch 955 BU at 77°C and corn starch 500 BU at 91.5°C. Addition of enzyme in amount of 120 NU/g, provokes the decrease of maximum viscosity. In comparison with viscosity of tested starches without enzyme, maximum viscosity with addition enzyme for tapioca decreases 38 times, potato starch 46 times and corn starch 2 times.

Viscosity of the hydrolyzates was also measured using Rheotest viscometer. Measurements were made for the hydrolyzates obtained within 1 h action of different enzyme dosages and at the duration of reaction within 3 h with application of enzyme in amount of 145 NU/g d.s. (Fig. 5) The increase of enzyme dose from 60-145 NU/g d.s. decreases their viscosity 5 times. Viscosity of maltodextrin is very important parameter in many fields of their application[Dziedzak 1988, Griffin and Brooks 1989, Luallen 1988, Schenck 1996, Summerkamp and Hesser 1990, Wang and Wang 2000].

Starch hydrolyzates obtained from potato starch characterize medium parameters (filtration rate and viscosity) among the tested starches. For bringing properties of tapioca and corn hydrolyzates closer to properties of potato hydrolyzate they are used in different proportions for their production (Fig. 6). The research indicates a possibility of decreasing the hydrolyzate viscosity by proportional increase of tapioca starch and proportional decrease of corn starch. Additionally, lower percentage contribution of tapioca starch in hydrolysed substrate influences positively the decrease of colour of the obtained hydrolyzate.

Application of tapioca and corn starches composition in comparison with potato starch for maltodextrin production decreased their production costs without decreasing the product's quality.

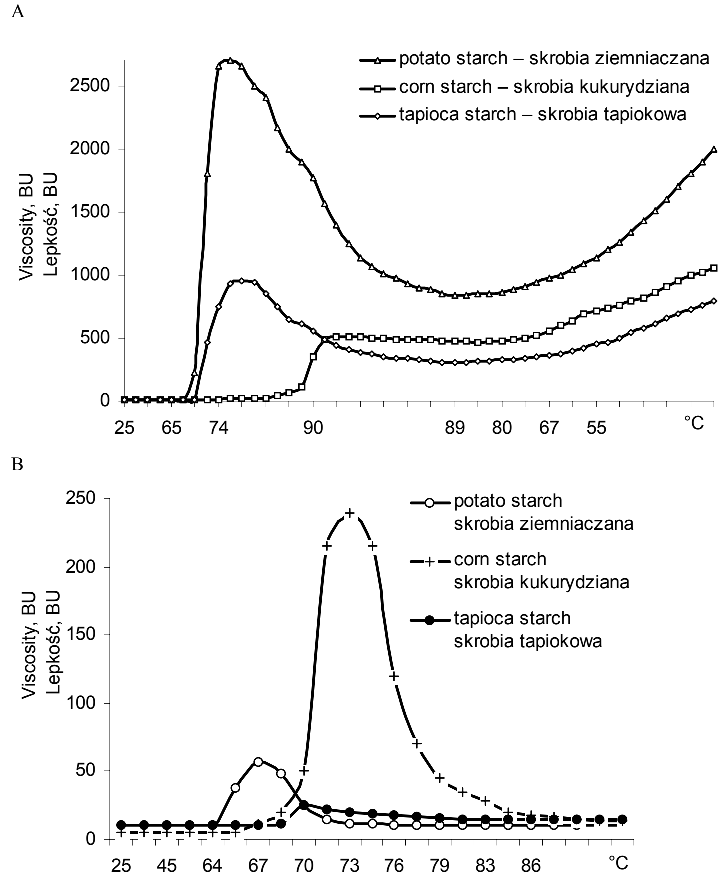

Fig. 4. Viscosity changes of starches, A – without the enzyme addition, starch concentration – 7.7% d.s., B – with the enzyme addition, starch concentration – 20% d.s., enzyme dosage 120 NU/g d.s.
Rys. 4. Zmiany lepkości skrobi, A – bez dodatku enzymu, stężenie skrobi – 7,7% s.s., B – z dodatkiem enzymu, stężenie skrobi – 20% s.s., dawka enzymu 120 NU/g s.s.

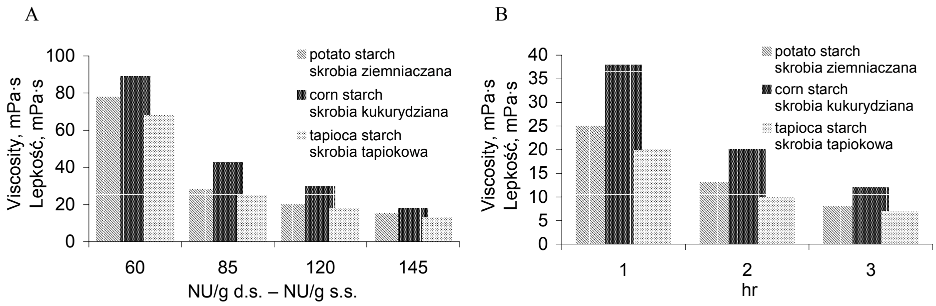

Fig. 5. Influence of the starch source on the viscosity value, A – dependence on the enzyme dosage, B – dependence on the hydrolysis time. Liquefaction conditions: 35% d.s. starch, pH – 5.9, temp. 95°C, A – time 1 h, B – enzyme dosage 145 NU/g d.s.
Rys. 5. Wpływ źródła skrobi na lepkość, A – wpływ dawki enzymu, B – wpływ czasu hydrolizy. Warunki upłynniania: 35-procentowa zawiesina skrobi, pH – 5,9, temp. 95°C, A – czas 1 h, B – dawka enzymu 145 NU/g s.s.

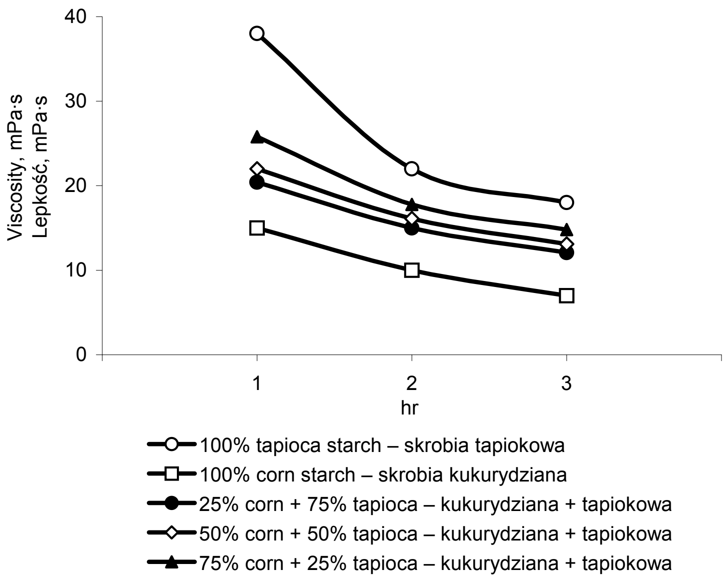

Fig. 6. Influence of the starches proportional contribution on the viscosity value of the liquefied starches. Liquefaction conditions: 35% DS starch, enzyme dosage 145 NU/g DS, pH – 5.9, temp. 95°C
Rys. 6. Wpływ udziału skrobi na lepkość upłynnionej skrobi. Warunki upłynniania: 35-procentowa zawiesina skrobi, dawka enzymu 145 NU/g s.s., pH – 5,9, temp. 95°C

CONCLUSIONS

1. Termamyl LS action influences similarly the progress of hydrolysis and carbohydrate composition in spite of different kind of starches used for hydrolyzate production.

2. The source of the investigated starches has influence on the viscosity values of the obtained hydrolyzates that make the differences in their filterability.

3. The application of the tapioca and corn starches mixture as a substrate for Termamyl LS action gives similar viscosity to the viscosity of the potato starch hydrolyzates.

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UPŁYNNIANIE SKROBI TERMOSTABILNĄ ALFA-AMYLAZĄ

Streszczenie: W hydrolizatach skrobiowych, w wyniku działania termostabilną alfa--amylazą na skrobię (ziemniaczaną, kukurydzianą, tapiokową), wykonywano pomiar wartości współczynnika glukozowego, składu węglowodanowego, lepkości i szybkości filtracji. Różnice podatności badanych skrobi na działanie Termamylu 120 LS są niewielkie (mniej niż 2 DE). Skład węglowodanowy hydrolizatów o zbliżonym DE, uzyskanych z różnych skrobi, jest podobny. Lepkość hydrolizatów otrzymywanych z mieszaniny skrobi tapiokowej i kukurydzianej jest bliska lepkości hydrolizatów uzyskanych ze skrobi ziemniaczanej.

Słowa kluczowe: skrobia, alfa-amylaza, hydrolizat, szybkość filtracji, lepkość

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