PHYSICAL AND CHEMICAL MODIFICATION OF POTATO STARCH TO OBTAIN RESISTANT STARCH PREPARATIONS

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The aim of the study was to obtain soluble and extruded starch preparations from potato starch, then to subject them to roasting with phosphates and/or glycin as well as to assess the effect of the order of double chemical modifications on their properties. The soluble starch was prepared by Lintner method, whereas the extruded starch was obtained using a single-screw extruder at 140-160-180°C. The starch preparations were produced using single chemical modification (roasting at 160°C with phosphates or glycin) as well as double modification of starch with a various order of the processes applied. The content of phosphorus, water solubility at a temperature of 30 and 80°C, resistance to amyloglucosidase activity and flow curves of the gelatinised starches were determined. The modifications carried out caused considerable changes in starch properties, and direction and extent of those changes were found to depend on the type and order of modifications. The modifications allowed obtaining the preparations of soluble starch with the resistance to amyloglucosidase activity accounting for nearly 20%.

INTRODUCTION

Resistant starch is defined as a sum of starch and its degradation products which are not absorbed in the small intestine of a healthy man. Especially starch available for bacteria colonizing the colon is of outmost importance to health promoting properties, due to its ability to lower pH of the environment. The increased acidity of intestinal digesta is caused by bacterial fermentation of resistant starch and formation of short chain fatty acids, which affect the selection of colonic microflora, thus stimulating the growth of a number of bacteria groups, Bifidobacterium and Lactobacillus in particular, as well as eliminating or diminishing counts of pathogenic microorganisms. The fatty acids formed in the large intestine influence the organism's metabolism, particularly the synthesis of cholesterol and triglycerides. Butyric acid produced in the distal section of the gastrointestinal tract plays a key role in the prevention of colonic and rectal cancer. Food products containing resistant starch, in comparison to those rich in fully digestible starch, cause slower release of glucose after meal intake and, in consequence, reduce insulin response, which is beneficial to diabetic patients. Reduction in the calorific value of food (the so-called "low calorie food"), prolonged sensation of satiety as a result of slow blood glucose release as well as increased intestinal filling evoke by resistant starch make it useful in diets for overweight patients [Zięba, 2009].

Various types of resistant starch (RS) can be distinguished: RS 1 – starch physically inaccessible for digestive enzymes, *e.g.* starch of partly milled cereal grains; RS 2 – starch resistant to the activity of amylases, non-gelatinised starch of some plant species, *e.g.* granules of potato starch; RS 3 – retrograded starch, *e.g.* from cooled cooked potatoes; and RS 4 – physically or chemically modified starch resistant to the activity of amylases [Englyst *et al.,* 1992].

One of the methods of resistant starch preparation is its partial depolymerisation followed by chemical modification. The division of a starch chain may occur due to chemical (acids and bases), biochemical (amylolytic enzymes) or physical (extrusion, thermolysis) factors [Englyst *et al.,* 1992]. The chemical modifications increasing starch resistance include hydroxypropylation, monophosphate formation, heating with glycin or iron ions [Leszczyński, 2004], and acetylation [Thanh *et al*., 2007].

The aim of the study was to produce soluble and extruded starch preparations, subsequently modified by phosphorylation and/or heating with glycin as well as to assess the effect of the order of double chemical modification on their selected properties.

MATERIALS AND METHODS

Production of preparations

Soluble (S) and extruded (E) starch preparations were produced from potato starch. Potato starch (PEPEES Łomża) with moisture content of 25% was extruded in a single-screw laboratory extruder DN 20 (Brabender) at 140-160-180°C. Soluble starch was prepared by Lintner method, by keeping the suspension of potato starch in 7.5% hydrochloric acid (1:5) for 7 days at room temperature, washing with distilled water and 0.5% $NaHCO_3$ and then drying [Richter *et al.*, 1968].

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Soluble and extruded starch preparations were subjected to roasting with a mixture of phosphates or with glycin (Figure 1). Subsequently, the modified preparations obtained were roasted again with phosphates or glycin in order to obtain doubly modified starch (Spg, Sgp, Epg, Egp). The reference samples were roasted, chemically non-modified preparations.

In order to carry out the phosphorylation, an aqueous solution of phosphates was mixed with starch or modified starch preparations, dried at a temperature of 60°C for 12 h and roasted at 160°C for 3 h. The modified starch was rinsed with ethyl alcohol at 1:3 ratio and dried at 30°C [Zięba *et al.*, 2007a].

Heating with glycin was run as follows: an aqueous solution of glycin was mixed with starch or modified starch preparations and then handled analogously to the esterification process [Kapelko *et al.*, 2007].

Analyses

The resultant preparations of extruded (E) and soluble (S) starch, and modified starch preparations were analysed for:
• content of phosphorus by the spectrophotometric method according to the Polish Standard [PN-EN ISO 3946:1994] after wet combustion in a microwave system for sample preparation Mars 5 (CEM Corporation) [PN-EN 13805:2003];
• water solubility at a temperature of 30 or 80°C [Richter *et al.*, 1968];
• flow curves of 5% pastes at a temperature of 50°C with the use of an RS 100 rotary viscosimeter (Haake), at a shear rate of $1-300$ s^{-1}, which were described by the Ostwald equation [Zięba *et al.*, 2007b];
• susceptibility of the preparations to the activity of amyloglucosidase (amigase by Genecor) after gelatinisation at a temperature of 100°C. The temperature of hydrolysis was 37°C and the concentration of enzyme was adjusted so that saccharification of gelatinised native starch occurred after 120 min of the process. The content of free glucose was determined colorimetrically at a wavelength of $\lambda=500$ nm, using a reagent for glucose concentration assay containing glucose oxidase and peroxidase (Biosystem). Saccharification was claimed to be complete when two subsequent results, obtained every hour, did not differ between one another [Zięba *et al.*, 2007b].

The results were subjected to a statistical analysis using an analysis of variance at a confidence level of $p<0.05$ [Stanisz, 2001].

RESULTS AND DISCUSSION

The content of phosphorus in native starch depends mainly on the type of starch, and in the case of potato starch
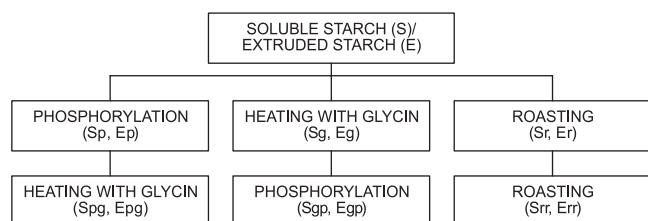
it oscillates from 60 to 90 mg/100 g [Teege, 1984]. In monophosphates obtained from potato starch by roasting for 3 h at a temperature of 160°C it accounts for *ca.* 800 mg/100 g [Fortuna, 1994]. The content of phosphorus in non-phosphorylated starch preparations ranged from 56 to 62 mg/100 g for soluble starch and from 70 to 75 mg/100 g for extruded starch (Table 1). The phosphorylation caused a multiple (nine- to twelvefold) increase in phosphorous content in the starch preparations. The highest content of this element was noticed in crude monophosphates (Sp – 709 mg/100 g, Ep – 775 mg/100 g). Roasting of starch with phosphates and glycin significantly diminished the content of phosphorous, and the extent of those changes was affected by the order of modifications applied. The content of phosphorus in the Spg preparation was higher by 14% than that reported in the Sgp preparation, whereas in the Epg preparation – higher by 19% in comparison to the Egp preparation. Presumably, the decrease in phosphorous content in starch preparations roasted with glycin is due to the partial blocking of phosphate groups access to starch by Maillard reaction products formed during roasting with glycin.

Water solubility depends on, among other things, the type of starch [Fortuna, 1994], the method of its modification and the temperature of measurement. All preparations of modified starch were characterised by substantial water solubility at

TABLE 1. Content of phosphorus in starch preparations.

Preparation type	Content of phosphorus (mg/100 g)	
S	59.88	
Sr	57.21	
Sp	708.67	
Sg	61.57	
Srr	56.48	LSD = 3.21
Spg	667.34	
Sgp	583.29	
E	74.83	
Er	72.81	
Ep	775.28	
Eg	72.31	
Err	70.37	
Epg	738.43	
Egp	621.25	
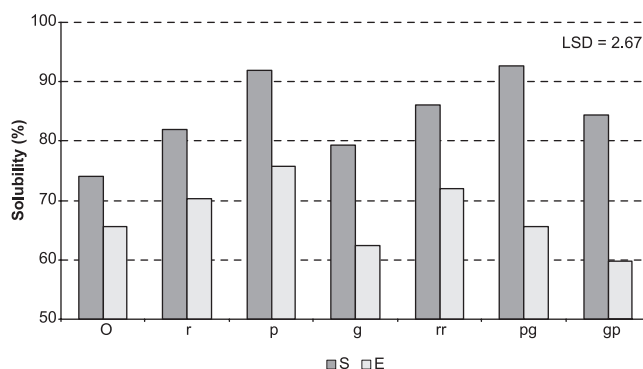
FIGURE 2. Water solubility of starch preparations determined at 30°C.

FIGURE 1. Modification procedure of starch preparations.

a temperature of 30°C (Figure 2). The solubility of the chemically unmodified soluble starch preparation accounted for 75%, and that of extruded starch for 66%. The roasting was noticed to increase the solubility of extruded starch only to a small extent, and the solubility of soluble starch preparations by 10%. The phosphorylation of the studied preparations resulted in their increased solubility in water. The Sp monophosphate was characterised by water solubility of 92%, whereas Ep of about 76%. A similar relationship was noticed by Fortuna [1994] in starch monophosphates obtained from starches of different origin. An increase in the number of phosphate groups in the starch chain is accompanied by the disruption of internal bonds stabilising its crystalline structure, which can enhance the solubility of preparations. The heating with glycin resulted in diminished solubility of starch preparations. The Sg preparation was characterised by app. 80%, whereas Eg by 62% water solubility. This dependency was likely to result from Maillard compounds formation, which affected the solubility of the modified preparations [Kroh *et al.*, 1996]. Water solubility was also significantly affected by the order of modifications applied, namely greater solubility was noticed for the preparations phosphorylated and then roasted with glycin, than for those modified in the reversed order.

A similar tendency, however at a considerably higher solubility, was observed in the case of solubility determination at
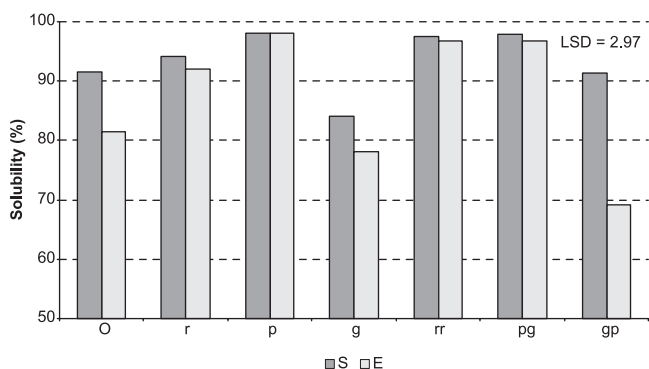
a temperature of 80°C (Figure 3). The solubility of the chemically unmodified soluble starch preparation accounted for 92%, while that of the extruded starch for 82%. Roasting was found to increase solubility value, which in preparations modified by roasting with phosphorus amounted to 98%. In contrast, roasting with glycin diminished solubility of the modified preparations by 80%. The order of modifications applied affected the studied feature to a significant extent. Much higher solubility was noticed for the preparations modified with phosphorous and then glycin than for those modified in the reversed order

Flow curves of the pastes prepared from the investigated starches are depicted in Figure 4. The flow curves of pastes prepared from soluble starch and its modified preparations did not display any distinct differences. At the maximum shear rate, the shear stress values of the analysed solutions were low

TABLE 2. Rheological parameters of prepared starch preparations.

Preparation type	Model of Ostwald de Waele		
	K [Pa s^n] *10^{-4}	n	R^2
S	17.35	1.436	0.994
Sr	16.51	1.403	0.995
Sp	17.77	1.376	0.996
Sg	17.52	1.342	0.993
Srr	17.79	1.372	0.995
Spg	16.89	1.400	0.995
Sgp	17.52	1.389	0.996
E	19.96	1.475	0.996
Er	17.47	1.399	0.998
Ep	14.08	1.635	0.995
Eg	16.50	1.462	0.996
Err	16.63	1.407	0.996
Epg	15.73	1.560	0.997
Egp	17.33	1.436	0.996

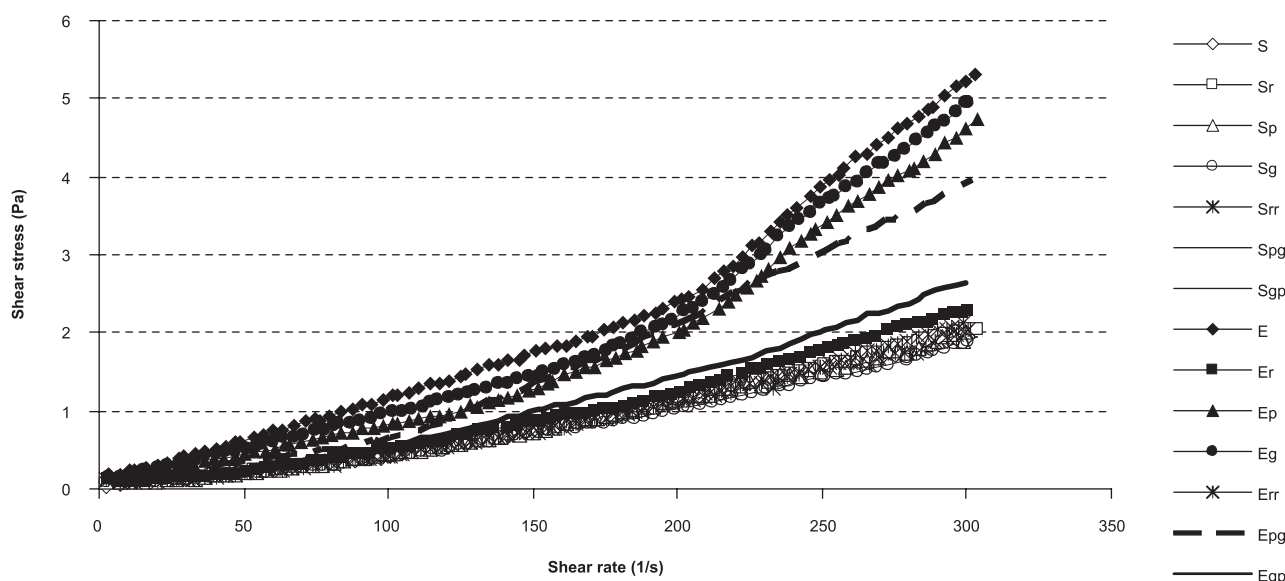FIGURE 3. Water solubility of starch preparations determined at 80°C.

FIGURE 4. Flow curves of pastes prepared from starch preparations.

TABLE 3. Saccharification degree of starch preparations.

Preparation type	Saccharification degree (%)	
S	99.42	
Sr	98.85	
Sp	91.57	
Sg	86.84	
Srr	84.04	
Spg	98.95	LSD = 1.75
Sgp	82.08	
E	99.62	
Er	98.85	
Ep	86.84	
Eg	96.40	
Err	91.47	
Epg	92.24	
Egp	94.41	

and ranged from 1.9 to 2.1 Pa. Considerably greater differences in the course of the flow curves were noticed for the second group of the analysed preparations. The paste obtained from extruded starch was characterised by the highest values of shear stress in the entire course of the flow curve, reaching 5.5 Pa at the maximum shear rate. Single and double roasting of this type of starch decreased twice the value of the shear stress recorded. It was caused by starch thermolysis proceeding during roasting [Lewandowicz, 2001]. Roasting of the extruded starch with phosphates or glycin increased shear stress values, in comparison to those of the roasted chemically unmodified starch. The shear stress value of the paste obtained from monophosphate at the maximum shear rate accounted for 4.7 Pa, whereas the one obtained from starch roasted with glycin for 8.0 Pa. Double chemical modification of extruded starch also increased shear stress value, and the order of modifications affected the course of the flow curves. The paste made of the extruded starch modified by phosphorylation and then roasting with glycin was characterised by higher shear stress values than the paste obtained from the starch modified in the reversed order. This is likely to result from differences in phosphorous content, which enhances viscosity of pastes to the greater extent than glycin [Zięba *et al.*, 2007a].

Parameters of the Ostwald-de-Waele exponential equation describing flow curves were compiled in Table 2. The determination coefficient $R^2 > 0.993$ proves good fitting of experimental data to the model applied. The n index, being a measure of deviation from the Newtonian flow, was much higher than unity (1.39-1.64). Therefore, the pastes obtained are of the shear thickening type. However, it is of no practical significance due to very low values of the shear stress, and also low viscosity of the analysed solutions. The consistency coefficient K, being a measure of paste viscosity in the initial phase of shearing, was very low and ranged from 0.0016 to 0.0110 Pa s^n.

The degrees of starch preparations saccharification with amyloglucosidase were presented in Table 3. The chemically unmodified soluble starch and extruded starch were more susceptible to the enzyme's activity; they were hydrolysed up to approximately 99%. Not complete enzymatic degradation of extruded starch has been confirmed by other authors [Ralet *et al.*, 1990; Unlu *et al.*, 1998], although some others have reported on the lack of the effect of the extrusion process on the formation of resistant starch [Faraj *et al.*, 2004]. Single roasting of the preparations did not evoke any substantial changes in their susceptibility to enzymatic hydrolysis. In turn, the re-roasting process significantly enhanced the resistance of starch to amyloglucosidase activity. The saccharification degree of Srr starch accounted for 84%, while that of Err starch for 91%. The influence of roasting on the amylolysis of starch was reported in other papers [Masłyk *et al.*, 2003]. Dextrins formed during thermolysis and free glucose bind to starch chains randomly at high temperature; it results in the formation of bonds between glucose residues that do not occur in native starch. That is why those bonds cannot be disrupted by amylolytic enzymes [Leszczyński, 2004]. Single modifications applied in the study decreased the starch susceptibility to amylases activity and their impact varied depending on the type of starch. The phosphorylation decreased the susceptibility to amylolysis more effectively in the case of extruded starch (Sp 92%, Ep 87%), whereas roasting with glycin – in the case of soluble starch (Sg 87%, Eg 96%). Double chemical modification carried out in various orders affected starch preparations to a different extent. Roasting with glycin of starch monophosphates enhanced, whereas phosphorylation of starch previously roasted with glycin diminished the susceptibility of the preparations to amylases in comparison to singly modified starch preparations. Unexpectedly, it was noticed that doubly modified starches (except of Sgp starch) were saccharified to the greater extent than re-roasted starch. It could have been caused by Maillard reaction and esterification that are claimed to block the formation of untypical 1-2 and 1-3 glycosidic bonds during the roasting of native starch.

CONCLUSIONS

1. The modifications applied affected properties of the obtained starch preparations, and the direction and extent of those changes depended on the type and order of modifications.

2. The content of phosphorous in starch not subjected to chemical modification ranged from 57 to 75 mg/100 g of starch. The phosphorylation caused *ca.* tenfold increase in phosphorous content of preparations, and a higher content was noticed in preparations roasted with phosphates and then with glycin, in comparison to those modified in the reversed order.

3. The phosphorylation caused an increase, while roasting with glycin a decrease in water solubility of starch, in comparison to the roasted chemically unmodified starch. The water solubility of doubly modified starch was affected by the order of modifications applied. A higher solubility was noticed for the preparations phosphorylated and then modified by roasting with glycin, than for the preparations modified in the reversed order.

4. All preparations produced were characterised by low viscosity of pastes formed, and the modifications of soluble starch carried out did not affect the course of flow curves. Roasting extruded starch diminished, whereas chemical mod-

ifications enhanced shear stress values in the entire course of flow curves.

5. The roasting and single chemical modifications diminished starch susceptibility to amylase activity, and the extent of their influence was different for various starch types. The roasting with glycine of starch monophosphates increased, whereas the phosphorylation of preparations previously roasted with glycine decreased the their susceptibility to amylase activity as compared to the singly modified starch.

ACKNOWLEDGEMENTS

REFERENCES

1. Englyst H.N., Kingman S.M., Cummings J.H., Classification and measurement of nutritionally important starch fractions. Eur. J. Clin. Nutr., 1992, 46, Suppl.2, 33–50.

2. Faraj A., Vasanthan T., Hoover R., The effect of extrusion cooking on resistant starch formation in waxy and regular barley flours. Food Res. Int., 2004, 37, 517–525.

3. Fortuna T., Studies on starch phosphates with a low degree of substitution with phosphorus. Habilitation Thesis No. 188. Zeszyty Naukowe Ar Kraków 1994 (in Polish).

4. Kapelko M., Zięba T., Properties of extruded potato starch modified with glycin. Żywność. Nauka. Technologia. Jakość, 2007, 54, 21–30.

5. Kroh L.W., Schumacher D., Untersuchungen zum Abbau von Maillard – Reaktion Produkten durch amylolytische Enzyme. Lebensm. Unters. Forsch., 1996, 203, 385–390.

6. Leszczyński W., Resistant starch – classification, structure, production. Pol. J. Food Nutr. Sci., 2004, 13/54, SI 1, 37–50.

7. Lewandowicz G., Modification of starch with the use of a microwave field. Habilitation Thesis No. 276, Zeszyty Naukowe Akademii Rolniczej w Krakowie, 2001 (in Polish).

8. Masłyk E., Leszczyński W., Gryszkin A., Modification–induced changes in potato starch susceptibility to amylolytic enzyme action. Pol. J. Food. Nutr. Sci., 2003, 12/53, SI 1, 54–56.

9. Polish Standard PN-EN 13805:2003. Food products. Determination of trace elements. Pressure mineralization (in Polish).

10. Polish Standard PN-EN ISO 3946:1994. Determination of the total phosphorus kontent with the spectrophotometric method (in Polish).

11. Ralet, M.-C., Thibault J.-F., Della Valle G., Influence of extrusion-cooking on the physico-chemical properties of wheat bran, J. Cereal Sci., 1990, 11, 249–259.

12. Richter M., Augustat S., Schierbaum F., Ausgewählte Methoden der Stärkechemie, VEB Fachbuchverlag Leipzig 1968.

13. Stanisz A. Przystępny kurs statystyki w oparciu o program Statistica PL na przykładach z medycyny. 2001, vol. 1, Statsoft Polska, Kraków (in Polish).

14. Teege G., Stärke und Stärkederivate. Behr's GmbH Co., 1984, Hamburg.

15. Thanh J.L., Błaszczak W., Lewandowicz G., Digestibility *vs.* structure of food grade modified starches. EJPAU, 2007, 10, 3.

16. Unlu, E., Faller J.F., Formation of resistant starch by a twin-screw extruder. Cereal Chem., 1998, 75, 346–350.

17. Zięba T., Resistant starch in food products. 2009, in: Potato III. Food 3 (Special Issue 1), (eds. Yee N., Bussel W.). Global Science Books, pp. 67–71.

18. Zięba T., Kapelko M., Jacewicz K., Styczyńska M., Properties of extruded starch modified with phosphorus and glycin. Pol. J. Food Nutr. Sci., 2007a, 57, 4 (C), 633–638.

19. Zięba T., Kapelko M., Gryszkin A. Selected properties of potato starch subjected to multiple physical and chemical modifications. Pol. J., Food Nutr. Sci., 2007b, 57, 4(C), 639–645.