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PROPERTIES OF EXTRUDED STARCH MODIFIED WITH PHOSPHORUS AND GLYCIN

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Key words: potato starch, extrusion process, starch monophosphate, glycin

The research was aimed at determining properties of starch preparations obtained from starch extruded at various temperatures and subjected to roasting either with phosphates or with phosphates and glycin. Determinations were carried out for: colour difference, content of phosphorus, thermal characteristics of gelatinization DSC, water absorption and water solubility, viscosity, susceptibility of the preparations to the activity of amyloglucosidase. Modification of starch with phosphorus affected a considerable attenuation of differences in some properties resulting from various temperatures of the extrusion process as compared to starch not subjected to chemical modification. The roasting of starch extruded with phosphates and glycin caused its considerable darkening (brown colour) and reduced water absorption determined under cold conditions as compared to starch roasted only with phosphates. The modification with phosphates and glycin did not evoke the expected change in the susceptibility of preparations to the activity of amyloglucosidase.

In the present study, monophosphates obtained from extruded potato starch were characterised by *ca*. 30% resistance to the activity of amyloglucosidase and high (*ca*. 90%) water solubility.

INTRODUCTION

Material subjected to the extrusion process is exposed to the activity of high temperature, mechanical forces and elevated pressure. In a short period of time, starch is transformed into plastic mass and then, as a result of a rapid pressure decrease and water evaporation, a product with a characteristic texture is obtained. During that process, the structure of starch granules is disrupted and the degree of crystallinity and its spatial arrangement are observed to change. Extrusion evokes an increase of water solubility and a decrease of viscosity of starch pastes [Jamroz *et al*., 1998] as well as diminishes susceptibility of starch preparations to the activity of amylases [Unlu & Faller, 1998]. Products of chemical modification of starch characterized by solubility in cold water [Lewandowicz, 2001] and reduced susceptibility to the activity of amylases [Masłyk *et al*., 2003] are starch monophosphates. The degree of substitution with phosphates, hence the properties of the resultant modified preparation, are mainly determined by the moisture content and origin of starch as well as time and temperature of its roasting [Fortuna, 1994]. The reduced susceptibility of starch to enzymatic activity results from changes in its structure [Leszczyński, 2004]. Roasting of starch with phosphates or glycin leads to the production of esters and Maillard-type compounds that impair the access of an enzyme to a starch chain [Gryszkin *et al*., 2004a]. A combination of physical modification (extrusion) with chemical one (roasting with phosphates or with phosphates and glycin) may lead to the production of starch preparations with a high water solubility and reduced susceptibility to amylolytic decomposition.

The research was aimed at determining properties of starch preparations obtained from starch extruded at various temperatures and subjected to roasting either with phosphates or with phosphates and glycin.

MATERIAL AND METHODS

Production of preparations. Potato starch with moisture content of 25% was extruded in a single-screw laboratory extruder DN 20 (Brabender) at the following temperatures 50-60-70°C, 100-110-120°C and 150-160-170°C, next ground and sieved through a screen with mesh size of 400 μm. Three preparations of extruded starch obtained in this way were divided into three portions: the first was roasted at a temperature of 160°C for 3 h, the second was modified with phosphorus, and the third was modified with phosphorus and glycin. In order to obtain starch monophosphates, an aqueous solution (100 mL) of sodium phosphates (10.44 g $NaH_2PO_4 \times H_2O$ and 52.54 g $NaHPO_4 \times 12H_2O$) was thoroughly mixed with 200 g of extruded starch, dried at a temperature of 60°C for 12 h to a moisture content of *ca.* 10%, and roasted at a temperature of 160°C for 3 h. The third portion was subjected to a combined action of phosphorus and glycin. An aqueous solution of glycin (20 g of glycin) and a solution of sodium phosphates (10.44 g $Na_2H_2PO_4 \times H_2O$ and 52.54 g $NaHPO_4 \times 12H_2O$) were mixed with 200 g of extruded starch, and then dried and roasted as described above for starch monophosphate. To re-

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move residues of reagents, all preparations were rinsed successively with three portions (300 mL each) of 60% and one portion of 96% ethyl alcohol. The modified preparations were dried at a room temperature and sieved through a screen with mesh size of 400 μm.

Analyses. The obtained preparations of extruded and roasted starch (E70p, E120p, E170p), extruded and phosphate-modified starch (E70f, E120f, E170f), and those extruded and modified with phosphates and glycin (E70fg, E120fg, E170fg) were determined for:

(1) colour difference (darkening) ΔE, calculated from Hunter colour scale values (L, a, b) and determined with a Minolta CR–200 chronometer in reference to native starch. Colour difference was calculated with the following formula:

$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$ [Clydesdale, 1978];

(2) content of phosphorus with the spectrophotometric method according to the Polish Standard [PN-EN ISO 3946:1994] after dry-combustion of a microwave system for sample preparation Mars 5 (CEM Corporation) [PN-EN ISO 13805:2003];

(3) thermal characteristics of gelatinization with the use of a differential scanning calorimeter DSC 822E (Mettler Toledo), in a temperature range of 25–100°C and heating rate of 10°C/min. The analysis was carried out in ME51119872 vessels using *ca*. 30-mg weighed portions of starch. Redistilled water was added to the weighed samples at a ratio of 3:1. Having closed the vessels, the samples were conditioned for 24 h at a room temperature and measurements were performed in respect of en empty reference vessel;

(4) water absorption and water solubility at a temperature of 30° or 80°C [Richter *et al*., 1968];

(5) viscosity (η_{50}) of 5% pastes at a temperature of 50°C read out from a flow curve ($\gamma = 0 \div 300$) plotted with the use of a rotary viscometer RS 100 (Haake) at a shear rate of 50 s^{-1}, using coaxial cylinders (Z38) with a single clearance as a measuring element;

(6) susceptibility of the preparations to the activity of amyloglucosidase (amigase by Genecor). The content of free glucose was determined colorimetrically using a reagent for glucose concentration assay by Biosystem that contained glucose oxidase and peroxidase. The analysis was conducted as follows: acetate buffer with pH=4.5 was added to a suspension of the starch preparation. The flask was fixed in a water bath of a shaker at a temperature of 37°C and supplemented with an enzyme solution. The concentration of enzyme was adjusted so that the complete saccharification of gelatinized natural starch occurred after 120 min of the process. Each hour, 5 mL samples of hydrolysate were collected for centrifugation. From the centrifuged sample, supernatant was collected that was next with a reagent by Biosystem, incubated at a temperature of 20°C for 15 min and measured for absorbance by means of a CECIL CE 2010 colorimeter at a wavelength of λ=500 nm. Measurements were performed against a blank sample, *i.e.* reagent with acetate buffer. The content of glucose was read out from a curve plotted as above using analytically pure glucose solutions. Saccharification was computed in respect of the theoretical content of glucose produced from the total saccharification of a weighed portion of starch. The result of hydrolysis was claimed final when three subsequent results did not differ between one another.

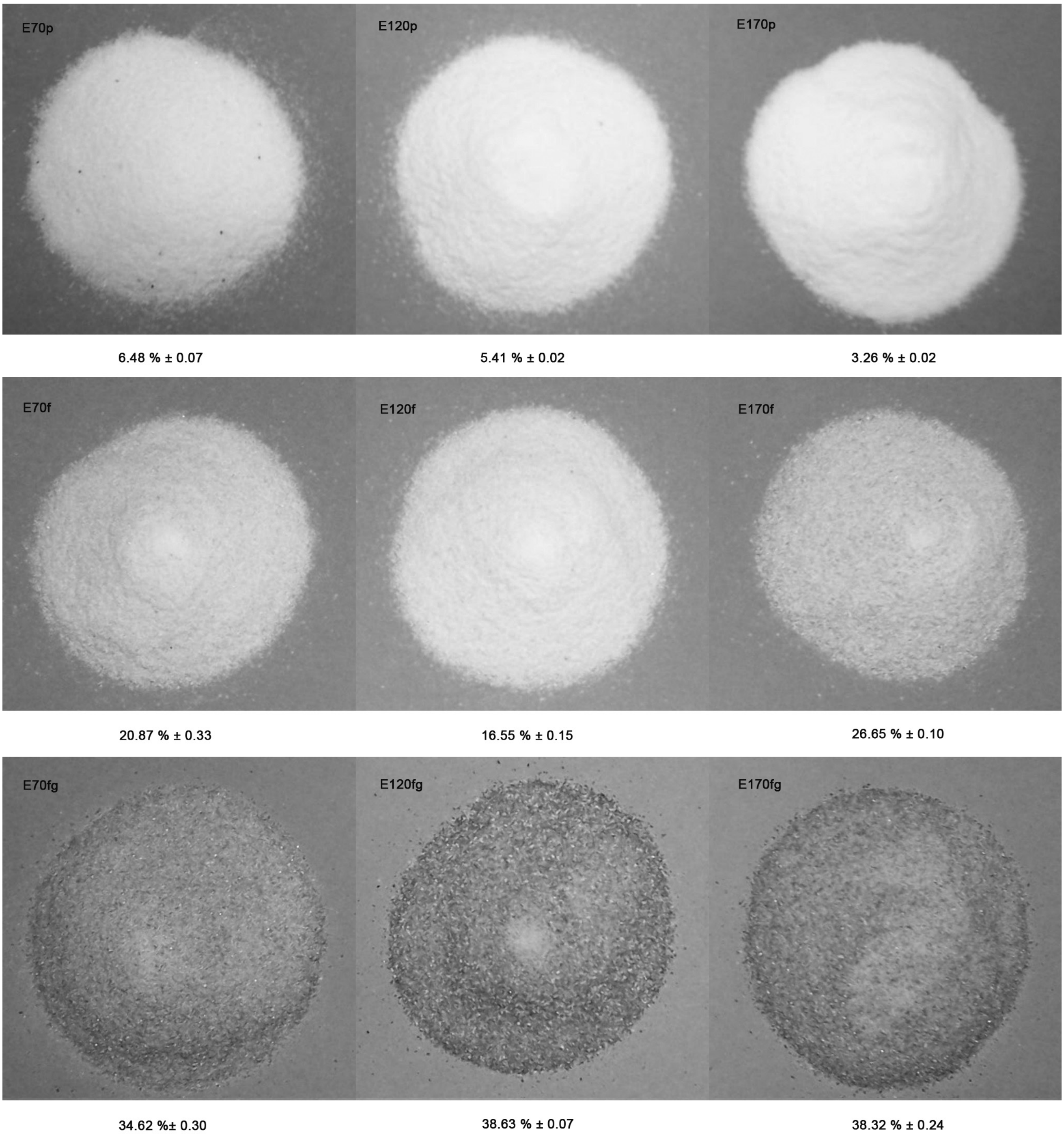
Statistical analysis. The results were elaborated statistically using an analysis of variance at a confidence level of $p<0.05$ [Stanisz, 2001].

RESULTS AND DISCUSSION

Extruded and then roasted starch was characterised by slightly darker colour than the native starch. What is more, the following tendency was observed: the lower the extrusion temperature the more distinct the difference. Extrusion at a temperature of 150-160-170°C caused darkening of starch by 3.5%, and that carried out at a temperature of 50-60-70°C by 6.5% (Photo 1). Roasting with phosphates evoked a change in the colour of starch preparations into pale yellow typical of starch monophosphates. It was reflected in ΔE coefficient ranging from 16 to 27%. The greatest change of colour could be observed in preparations roasted with phosphates and glycin which demonstrated brown colour with a darkening coefficient of ΔE 34-39%. Such a substantial alternation of colour indicates that during starch roasting with glycin a Maillard reaction occurs that produces a characteristic brown colour [Kramhöller *et al.,* 1993].

Roasting with phosphates as well as with phosphates and glycin affected *ca*. tenfold increase in phosphorus content of the preparations as compared to the preparations of starch not subjected to chemical modification (Figure 1). The content of phosphorus in roasted preparations ranged from 73 to 83 mg/100 g, and in those roasted either with phosphates or phosphates and glycin – from 775 to 854 mg/100 g of starch. The concentration of phosphorus in native starch is determined mainly by the type of starch, *e.g.* in potato starch it fluctuates between 60 and 90 mg/100 g [Teege, 1984], whereas 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 of starch [Fortuna, 1994].

Thermal DSC characteristics of the starch preparations demonstrated the presence of starch fractions whose transition in water proceeded in a wide range of temperatures at a relatively low heat of transition (Table 1). The range of temperatures and transition heat depended on the modification application. The greatest heat of transition was reported for preparations of extruded and roasted starch (3.0÷4.9 J/g), the increasing values of heat were observed at increasing temperatures of extrusion. Also the initial and final temperatures appeared to be determined by temperature of the extrusion process: the higher the extrusion temperature, the higher the initial temperature (47.0÷48.6°C) and the lower the final temperature (81.9÷79.9°C) of transition. An increase in heat at a decreasing range of transition temperatures evoked and increase in the mean specific heat (peak height index) that increased from 0.09 to 0.16 J/g°C along with increasing temperature of extrusion. The chemical modification resulted in a decrease of specific heat to a level of 0.05÷0.08 J/g°C and in *ca*. twofold decline in heat of starch transition that fluctuated between 1.2 and 2.4 J/g and was observed to increase at elevating extrusion temperatures. The initial temperature of



6.48 % ± 0.07 5.41 % ± 0.02 3.26 % ± 0.02

20.87 % ± 0.33 16.55 % ± 0.15 26.65 % ± 0.10

34.62 %± 0.30 38.63 % ± 0.07 38.32 % ± 0.24

PHOTO 1. Photos and colour difference ΔE of starch preparations in respect of native starch.

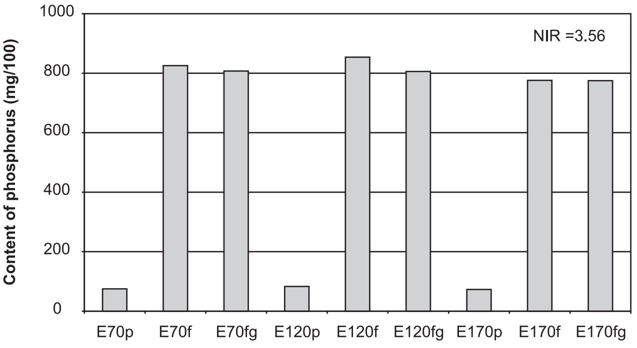


FIGURE 1. Content of phosphorus in starch preparations.

transition ranged from 47.3 to 49.5°C. The final temperature, which was higher in the case of starch extruded at higher temperatures, ranged from 71.4 to 79.6°C. The value of starch transition heat is determined by damage of the structure of starch chains arranged in double helices. In turn, the increasing range of temperatures, exerting an inversely proportional effect on the value of mean specific heat of transition, has been found to be affected by degradation of crystalline structures of starch [Singh & Singh, 2001]. Once treating extruded starch as a sort of starch paste with a very high concentration, it may be supposed that slow reconstruction of spiral structures and recrystallization of starch proceed in it. Simi-

TABLE 1. Temperatures of gelatinization, heat of transition and mean specific heat of starch preparations determined from DSC thermal characteristics.

Preparation type	Initial temperature (°C)	Final temperature (°C)	Heat of transition (J/g)	Mean specific heat (J/g* °C)
E70p	47.0 ± 0.1	81.9 ± 1.1	3.0 ± 0.01	0.09
E70f	47.7 ± 0.1	71.4 ± 0.1	1.2 ± 0.01	0.08
E70fg	47.9 ± 0.2	72.8 ± 2.9	1.5 ± 0.30	0.06
E120p	47.9 ± 0.1	82.5 ± 0.1	3.7 ± 0.03	0.11
E120f	49.5 ± 0.6	76.2 ± 0.9	1.4 ± 0.04	0.05
E120fg	49.5 ± 0.1	75.3 ± 1.6	1.5 ± 0.04	0.06
E170p	48.6 ± 0.2	79.9 ± 1.2	4.9 ± 0.23	0.16
E170f	47.3 ± 1.2	77.9 ± 1.8	1.9 ± 0.56	0.07
E170fg	48.0 ± 0.7	79.6 ± 0.2	2.4± 0.18	0.07

lar conclusions were drawn by Pirkko and co-workers [1999] based on thermal DSC characteristics of stored extruded starch. During its storage, an extended peak appeared that was characterized by a low heat of transition. The observed changes may be intensified by the roasting of extruded starch. The heating of starch results in depolymerization, transglucosidation and repolymerization processes occurring inside its molecule [Leszczyński, 2004]. Elongation of the time of the dextrinization process is accompanied by an increase in the number of, untypical to starch, 1,3 and 1,2 bonds between glucose residues of produced dextrins [Ohkuma *et al*., 1990].

Water solubility of extruded and roasted starch was observed to increase along with an increasing temperature of extrusion (Figure 2). Water solubility determined under cold conditions (at a temperature of 30°C) ranged from 46.3 to 71.1%, whereas that determined under hot conditions (at a temperature of 80°C) from 64.2 to 83.2%. Starch modification with phosphorus affected a decrease in differences in its water solubility resulting from different temperatures of extrusion. Water solubility assayed under cold conditions ranged from 47 to 60%, whereas that assayed under hot conditions accounted for *ca*. 84%, irrespective of the temperature of starch extrusion. A similar tendency may be observed in the case of preparations modified with phosphorus and glycin. Their solubility determined at a temperature of 30°C fluctuated between 51.4 and 55.7%, and that determined at a temperature of 80°C between 80.9 and 93.4%. An exception was E170fg preparation, whose solubility assayed under cold conditions reached 36.9%. Extruded starch is characterised by substantially higher water solubility than native starch, and the extent of those changes is determined, most of all, by the type of starch, its moisture content before extrusion and its parameters, especially temperature [Willet & Shogren, 2002]. High water solubility, dependent mainly on the time and temperature of roasting, is also typical of starch monophosphates [Fortuna, 1994]. A combination of both modifications resulted in the production of preparations with a considerable water solubility.

Water absorption of extruded and roasted starch determined at a temperature of 30 or 80°C ranged from 3.5 g to 10.2 g of water per 1 g of starch (Figure 3). Phosphorylation of starch caused a significant increase of water absorption determined under cold conditions. Extruded starch roasted with phosphates at the lowest temperature (50-60-70°C) was characterised by *ca*. 2 times, whereas that extruded at higher temperatures (100-110-120 and 150-160-170°C) by *ca*. 5 times higher water absorption than the starch not modified chemically. The high water absorption of starch monophosphates has been confirmed in a number of research papers [Gryszkin *et al*., 2004b]. The capacity for water absorption determined under hot conditions was considerably lower and reached a few per cents. This is probably linked with a high, 80-90%, solubility of the starch monophosphates formed. The roasting of starch with phosphates and glycin resulted in a diminished (by 22-32%) capacity for water absorption at a temperature of 30°C as compared to starch monophosphates. The modification of starch had no significant effect on its, anyway, low water absorption assayed under hot conditions.

Roasted extruded starch formed pastes with a low viscosity (0.029÷0.06 mPa·s) which was observed to decrease along with an increase in temperature of extrusion (Figure 4). That dependency results from intensive degradation of starch proceeding at higher temperatures of the extrusion process. The chemical modification of starch attenuated, to a significant extent, the effect of extrusion conditions on the viscosity of the prepared paste, which in the case of starch monophosphates ranged from 0.024 to 0.027 mPa·s, and in the case of preparations modified with phosphates and glycin – from 0.019 to 0.025 mPa·s. Lower viscosity of pastes prepared from starch modified with phosphates and glycin as compared to monophosphates most likely results from the hydrolytic action of

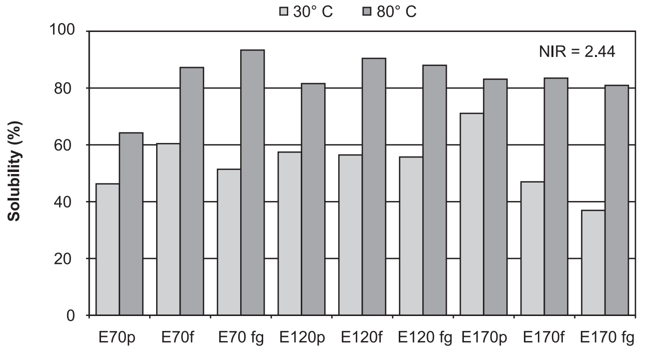


FIGURE 2. Water solubility of starch preparations determined at 30 and 80°C.

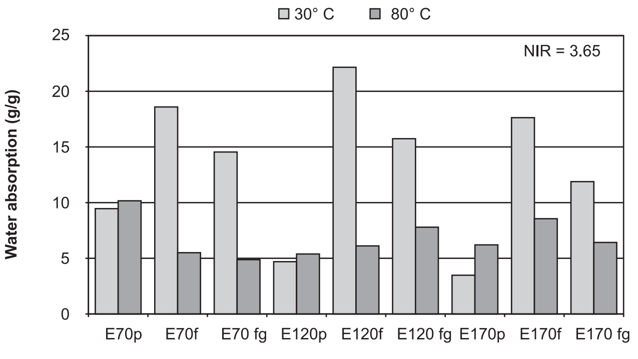


FIGURE 3. Water absorption of starch preparations determined at 30 and 80°C.

aminoactetic acid (glycin). One should also remember that the production of monophosphates is accompanied by concomitant thermolysis of starch that affects a reduction in viscosity as well as by phosphorylation that evokes an increase in the viscosity of pastes prepared [Lewandowicz, 2001]. Presumably, the equalization of the viscosity of the pastes examined was affected, to a much greater extent, by a similar and relatively high degree of substitution with phosphorus than differences in the molecular weight of starch extruded at various temperatures.

Susceptibility of chemically-unmodified starch to the activity of amyloglucosidase was found not to depend on extrusion temperature and reached *ca*. 93% (Figure 5). The produced starch monophosphates as well as starches roasted with phosphates and glycin were characterised by *ca*. 25% lower susceptibility to hydrolysis. Not complete enzymatic degradation of extruded starch has been confirmed in investigations of other authors [Ralet *et al*., 1990; Unlu & Faller, 1998], although some others have reported on a lack of the effect of the extrusion process on the formation of resistant starch [Faraj, 2004]. Diminished susceptibility of starch monophosphates to the activity of α-amylase, as compared to unmodified starches, has also been observed by Sitohy & Ramadan [2001], whereas *ca*. 20% resistance of starch monophosphates to the activity of amyloglucosidase has been confirmed by Masłyk and co-workers [2003]. Kroh & Schumacher [1996] claim that products of starch depolymerization roasted with glycin display a higher resistance to amylases. The simultaneous modification with phosphorus compounds and glycin did not evoke the expected change in the susceptibility of the produced preparations to the activity of amyloglucosidase.

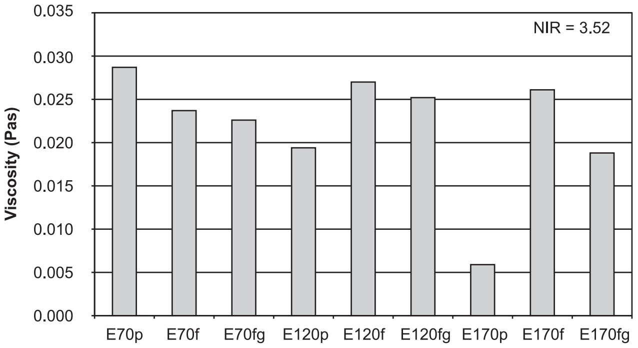


FIGURE 4. Viscosity η50 of 5% starch pastes at a temperature of 50°C.

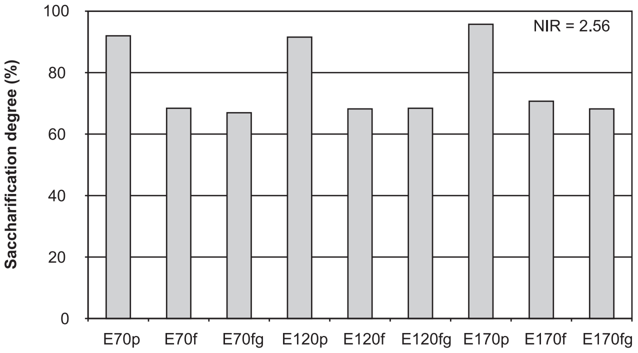


FIGURE 5. Saccharification of starch preparations.

CONCLUSIONS

The starch preparations examined appeared to differ in their properties, and the direction and extent of those changes depended on extrusion temperature and chemical modification applied.

The greatest differences were observed in extruded and roasted preparations whose properties were determined mainly by temperature of the extrusion process. Extruded starch roasted with phosphorus compounds had darker color (yellow), contained considerably more phosphorus, and was characterised by a lower heat of transition, several times higher water absorption determined under cold conditions and lower susceptibility to the activity of amylase, as compared to the extruded and roasted starch.

Modification of starch with phosphorus affected a considerable attenuation of differences in some properties resulting from various temperatures of the extrusion process as compared to starch not subjected to chemical modification. All starch monophospates were characterised by a similar heat and specific heat of transition, identical solubility assayed under hot conditions, similar solubility determined under cold conditions as well as similar water absorption determined under hot conditions, viscosity of the pastes prepared and the same susceptibility to the activity of amyloglucosiadses.

The roasting of starch extruded with phosphates and glycin caused its considerable darkening (brown colour) and reduced water absorption determined under cold conditions as compared to starch roasted only with phosphates. The modification with phosphates and glycin did not evoke the expected change in the susceptibility of preparations to the activity of amyloglucosidase.

In the present study, monophosphates obtained from extruded potato starch were characterised by *ca*. 30% resistance to the activity of amyloglucosidase and high (*ca*. 90%) water solubility.

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WŁAŚCIWOŚCI SKROBI EKSTRUDOWANEJ MODYFIKOWANEJ FOSFOREM I GLICYNĄ

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Celem pracy było określenie właściwości preparatów skrobiowych otrzymanych ze skrobi ekstrudowanej w różnych temperaturach, poddanych prażeniu z fosforanami lub prażeniu z fosforanami i glicyną. Wyznaczono: różnicę barwy, zawartość fosforu, charakterystykę termiczną kleikowania DSC, lepkość, podatność preparatów na działanie amyloglukozydazy. Badane preparaty skrobiowe różniły się swoimi właściwościami, a kierunek i wielkość tych zmian były uzależnione od temperatury ekstruzji oraz zastosowanej modyfikacji chemicznej. Modyfikacja skrobi fosforem wpłynęła na znaczne zmniejszenie różnic niektórych właściwości wynikających z różnej temperatury ekstruzji w porównaniu do skrobi nie modyfikowanej chemicznie. Prażenie skrobi ekstrudowanej z fosforanami i glicyną powodowało znaczne jej pociemnienie (kolor brązowy) i zmniejszenie wodochłonności oznaczonej na zimno w porównaniu do skrobi prażonej tylko z fosforanami. Przeprowadzona modyfikacja fosforanami i glicyną nie spowodowała spodziewanej zmiany w podatności preparatów na działanie amyloglukozydazy. W przeprowadzonych badaniach otrzymano monofosforany z ziemniaczanej skrobi ekstrudowanej o ok. 30% odporności na działanie amyloglukozydazy charakteryzujące się wysoką (ok. 90%) rozpuszczalnością w wodzie.