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PHOSPHORYLATED STARCH OF HIGH DEGREE OF SUBSTITUTION

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Starch phosphate of high degree of substitution may be prepared using phosphorus pentoxide either in pyridine or in benzene. Both these solvents act not only as the reaction medium but they also deteriorate the starch matrix.

Starch phosphates attract attention of producers of food as good gelating agents, sorbents and biologically active preparations. The preparation of starch phosphate of the high degree of substitution is a task of scientists [1]. Thus far reported methods of esterification of phosphoric acid with starch involve phosphorus acid and its salts as well as phosphorus oxychloride as phosphorylating agents. The degree of the substitution of starch with phosphoric acid groups is poor and does not exceed usually 0.1. Even if the degree of the substitution of the starch phosphate is low there is a chance of influencing of the properties of gels made of them by adjusting suitable reaction conditions [2, 3]. Salts of phosphoric, metaphosphoric and pyrophosphoric acids are compounds which poorly penetrate starch grains. Therefore, they may be used together with coreagents which loose the starch matrix. They are, for instance, alkali [4] or urea [5]. The addition of coreagents may be omitted by steaming of starch prior to reaction with phosphates [6]. Recently the phosphorylation of starch with phosphorus pentoxide has been reported and starch matrix has been loosed by preheating of starch prior to reaction with sodium carbonate [7]. In our work, the reaction of starch with the same phosphorylating agent is preceded by the deterioration of starch matrix by the effect of either pyridine or benzene.

MATERIALS AND METHODS

Oven-dried potato starch (Główno, 1989) (10 g) was placed in dry either benzene (50 mL) or pyridine (50 mL) folowed by addition of phosphorus pentoxide (1 to 20 g as shown in Table 1). The whole reaction mixture was stirred

for 1 h at room temperature. The temperature was gradually risen up to 80°C and maintained for 4 h. After the reaction was over the solvent was taken off by distillation under reduced pressure. The solid residue in form of granules was slowly transferred into cracked ice and alkaliied to pH 8 using saturated aq. NaHCO₃ solution. The solid was filtered off on sintered glass filter washed on the filter with 20-30% aq. ethanol then with pure ethanol and dried over molecular sieves.

Table 1. The phosphorus content in starch phosphates and degree of substitution with phosphorus acid moieties

Starch:P ₄ O ₁₀ (Reaction mixture)	Phosphorus content		Reaction conditions	Number of phosphorus moieties on 1 glucose unit
	organic [mg %]	inorganic [mg %]		
10: 1	172.7	44.30	Benzene, > 60°	0.028
10: 2	675.0	1170	as above	0.109
10: 3	1070	20.17	as above	0.172
10: 5	1835	423.2	as above	0.295
10: 5	4268	863.2	Benzene, 80°	0.687
10: 5	570.0	279.1	Pyridine, > 60°	0.09
10: 5	1273	220.3	Pyridine, 86°	0.205
10:10	2061	1843	Benzene, > 60°	0.33
10:20	6452	2162	as above	1.04
Native starch	73.43	0.81		

Organically bonded phosphorus and total phosphorus content were determined according to Marsh [8]. The results are presented in Table 1.

Samples of processed starch were characterized by means of characteristics of gelation (Rheotest II apparatus) in 7.2% aqueous suspensions. The speed of rotation of cylinder was $8 \text{ a} = 27 \text{ cycles/min}$.

Reaction with iodine was run according to [9] using Specord UVVIS spectrophotometer with 100 mm quartz. The measurement range was 250-800 nm.

Observation of samples under polarized light was carried out with BIOLAR FL fluorescent microscope 221, PZO Warszawa, Poland.

X-Ray debayograms were run using the TUR-62 + HZG apparatus, GDR with the copper lamp of $\lambda_{\text{CuK}\alpha} = 1.54178 \text{ \AA}$.

IR — absorption spectra were measured in the KBr pellets in 5.9 to 6.9% concentrations using SPECORD-IR Zeiss Jena spectrophotometer.

RESULTS AND DISCUSSION

As shown in Table 1 the phosphorylation of starch could be achieved with a high effectiveness. The content of phosphorus in esterified form exceeds by one two orders that in starch phosphates resulting from the use of phosphates as

phosphorylating agents. The calculations based on the content of bonded phosphorus point that in the most esterified starch each D-glucose unit carries one phosphorus acid moiety. The relevant data for other prepared starch phosphates are included in Table 1.

Benzene medium appears to be more suitable than pyridine. Pyridine which better solvates starch than benzene (the higher mutual affinity of both reagents) hinders the reaction sites of starch. The phosphorylation by described method does not affect the construction of the starch grains to any essential extent. The starch grains save their polarization crosses that may be seen under polarized light. However, as the content of bonded phosphorus in starch increases the polarization crosses cease. The gelation of grains which readily takes place in cold water is responsible for it. The characteristics of gelation show that benzene is more than pyridine matrix deteriorating solvent. Starch phosphorylated in pyridine exhibits a gelation curve whereas starch phosphorylated to a similar extent in benzene does not gelate but fully dissolves in water to give slightly viscous solution (Table 2).

Table 2. The characteristics of gelation of 7.2% aqueous suspensions of starch phosphates

Phosphorus content [mg%]	T_k [°C]	η_{max} [mPa.s]	T_{max} [°C]	η_{96} [mPa.s]	$\eta_{96/20}$	η_{50} [mPa.s]
127.7 ^a	58.5	1636.5	67	167	227	1273
675.0 ^a		below the sensitivity of the viscosimeter				
1835.0 ^a	60	42	80	18	18	42
2061.0 ^a		below the sensitivity of the viscosimeter				
4268.0 ^a		below the sensitivity of the viscosimeter				
6452.0 ^a		below the sensitivity of the viscosimeter				
570.0 ^b	62	108	72	18	6	24
1273.0 ^b	63.5	150	80	84	52	120
73.4 ^c	62	1940	73	72	168	1940

^a The phosphorylation in benzene.

^b The phosphorylation in pyridine.

^c The original starch before processing.

The phosphorylation with our method delivers products which barely slightly affects the gelation properties of original starch as long as the yield of phosphorylation is low (for instance, 172.7 mg%). More phosphorylated products, especially these prepared in benzene, form aqueous solutions of very low viscosity that the gelation characteristics could not be measured.

Table 3 presents the positions of the absorption maxima in the spectra of phosphorylated starch — iodine complexes.

They provide the calculation of an average length of the chains starch matrix. The results of the calculations are also included in this Table. One may see that the average length of the chains increases in respect to that in original

Table 3. The UVVIS characteristics of the starch phosphate complexes and the average length of the chains of D-glucose units.

Phosphorus content in the sample [mg%]	Maxima [nm] and their intensity	Number of D-glucose units in the chain
73.4	355.5 (.2566), 400.5 (.0902), 575.7 (.0867)	27.9
570.0	354.0 (.4027), 420.0 (.1423), 593.4 (.2635)	51.7
675.0	379.8 (2.26), 400.4 (1.186), 555.2 (.5712)	44.3
1835	377.5 (1.515), 425.7 (.7412), 575.7 (1.294)	59.5
2061	385.6 (.6442), 400.5 (.5366), 420.0 (.7850) 566.5 (.7850)	51.7
4422	356.0 (.4043), 378.1 (.3553), 577.0 (1.716)	60.8
6452	379.8 (2.14), 400.5 (1.446), 429.4 (1.179) 547.9 (1.537)	40.5

starch. It suggests that phosphorylation has a crosslinking character. Indeed, the phosphorylation has some influence on the ordering of the elements of the starch matrix as shown by debyograms of starch phosphates. The starch with the highest degree of the substitution is the least amorphous. The extent of crosslinking does not parallel to the increase of the phosphorus content. Thus original starch has the shortest chain and the most esterified starch have not got the longest chain. The degree of the esterification has undoubtedly some influence on the susceptibility of the preparation towards hydrolysis. The hydrolysis produces so-called hydrogen starch phosphate which is unstable on storage because of its further proton catalyzed autohydrolysis of glycosidic bonds [10]. Some evidence of susceptibility of our starch phosphates to the hydrolysis comes from the insight into IR absorption spectra.

There is well visible but broad peak centered at 1250 cm^{-1} in the spectra of freshly prepared starch phosphates. Its intensity in the spectra of dry starch phosphates increases with the content of bonded phosphores in starch phosphate. This broad peak is separated from a neighbouring peak at approximately 1160 cm^{-1} by a minimum at 1225 cm^{-1} . However, after leaving starch phosphate in the open air this minimum and the peak at 1250 cm^{-1} cease in favour of a broad curve descending from 1250 to 1160 cm^{-1} .

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Streszczenie

Ogrzewanie skrobi ziemniaczanej z bezwodnikiem kwasu fosforowego w benzenie lub pirydynie pozwala na otrzymywanie fosforanów skrobiowych o wysokim stopniu podstawienia. W najbardziej zestyfikowanym preparacie na jedną jednostkę glukozową przypada jedna reszta kwasu fosforowego. Estyfikacja ma charakter siecący. Otrzymane preparaty łatwo ulegają hydrolizie nawet w wilgotnym powietrzu. Kleiki skrobi fosforylowanej mają niższą lepkość aniżeli kleik ze skrobi wyjściowej.