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Short communication

Selected properties of acetylated adipate of retrograded starch☆

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ABSTRACT

Native potato starch (NS) and retrograded starch (R – obtained via freezing and defrosting of a starch paste) were used to prepare starch acetates: NS-A and R-A, and then acetylated distarch adipates: NS-ADA and R-ADA. The chemically-modified preparations produced from retrograded starch (R-A; R-ADA) were characterized by a higher degree of esterification compared to the modified preparations produced under the same conditions from native potato starch (NS-A; NS-ADA). Starch resistance to amylolysis was observed to increase (to 30–40 g/100 g) as a result of starch retrogradation and acetylation. Starch cross-linking had a significant impact on the increased viscosity of the paste in the entire course of pasting characteristics and on the increased values of rheological coefficients determined from the equations describing flow curves. The produced preparation of acetylated retrograded starch cross-linked with adipic acid (R-ADA) may be deemed an RS3/4 preparation to be used as a food thickening agent.

1. Introduction

Starch, which is a storage substance of many plants, is produced on the industrial scale mainly from maize, rice, potato, tapioca and wheat. The applicability of starch in the native form is low, but hydrolysates and modified preparations produced from it are widely used in practice (Zięba, 2009). One of the newest modified starch preparations used as a health-promoting food additive is resistant starch that occurs in various forms: RS1 – starch inaccessible to digestive enzymes, present in undigested plant cells; RS2 – non-pasted starch of selected plants (e.g. potato); RS3 – retrograded starch (Englyst, Kingman, & Cummings, 1992), RS4 – chemically- or physically-modified starch (Haralampu, 2000); and RS3/RS4 – retrograded chemically-modified starch (Zięba, Szumny, & Kapelko, 2011a). The name of the last type of starch was proposed after investigations of acetylated starch produced from retrograded potato starch (Kapelko, Zięba, Golachowski, & Gryszkin, 2012a; Kapelko, Zięba, & Michalski, 2012b; Zięba, Kapelko, & Gryszkin, 2007; Zięba, Juszczak & Gryszkin, 2011; Zięba et al., 2011a; Zięba, Szumny, & Kapelko, 2011b; Zięba, Kapelko, & Szumny, 2013). This type of starch is characterized by significant resistance to the action of amylases (ca. 40%) and – unlike preparations of RS3 starch available on the market – is capable of re-forming viscous pastes (Kapelko et al., 2012a; Zięba et al., 2011). The modification which has a positive impact on the rheological properties of prepared pastes is the cross-linking of starch with residues of adipic or phosphoric acid (Singh, Kaur, & McCarthy, 2007). The application of such modification to produce RS3/RS4 starch may increase the resistance and viscosity of pastes.

The aim of the present study was to produce acetylated adipate of retrograded starch via multi-stage modification of potato starch and to determine the effect of the applied modifications on selected properties of the resultant preparations.

2. Methodology

2.1. Materials

The initial experimental material included Superior Standard potato starch produced by PPZ Niechlów in 2010. Starch was modified with analytically pure acetic acid anhydride and analytically pure adipic acid, both purchased at POCH SA Gliwice, Poland.

2.2. Production of preparations

Starch preparations were produced according to the scheme presented in Fig. 1 (symbols used throughout the manuscript were provided in brackets in the Figure). Pastes were produced from native potato starch in the concentration of 10 g of starch per 100 g of solution, frozen at a temperature of −20 °C for 3 days and defrosted at a temperature of 20 °C for 2 days. The precipitated starch with a spongy structure was rinsed with distilled water, dried in an air dryer at a temperature of 30 °C for 24 h, ground and

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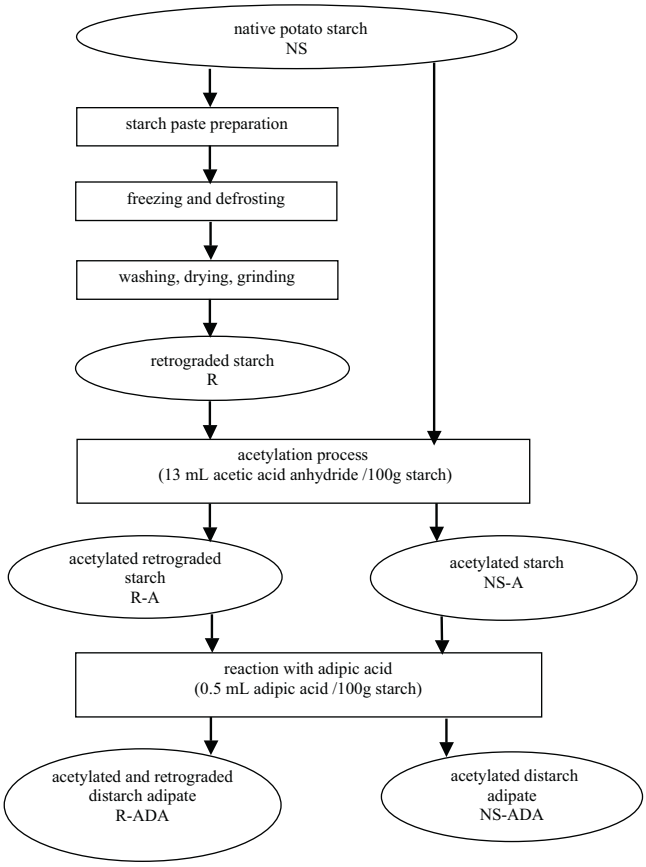


Fig. 1. Scheme of acetylated adipate production of retrograded or native starch.

sieved through a screen with a mesh diameter of 400 μm (Kapelko et al., 2012a).

Native starch (NS) and the produced preparation of retrograded starch (R) were acetylated with acetic acid anhydride under conditions provided by Kapelko et al. (2012a). The basic dose of acetic acid anhydride was adapted correspondingly to its quantity used in the acetylation process in the starch industry (13 mL/100 g starch).

The cross-linking of native starch and retrograded starch with adipic acid was conducted analogously to acetylation by instilling 1 mL of the cross-linking agent and hot-dissolving 20 g of adipic acid in 80 g of acetic anhydride.

The produced preparations of acetylated native starch (NS-A), acetylated native starch cross-linked with adipic acid (NS-ADA), acetylated retrograded starch (R-A), and acetylated retrograded starch cross-linked with adipic acid (R-ADA) were rinsed with distilled water, dried in an air dryer at a temperature of 30 °C for 24 h, ground and sieved through a screen with a mesh diameter of 400 μm.

2.3. Determination of the content of acetate groups

Ten grams of the preparations were poured into a conical flask, filled with 65 mL of distilled water, and neutralized by adding a few drops of 0.01 M NaOH in the presence of phenolphthalein until pale pink color was maintained for ca. 1 min. Next, 25 mL of 0.5 M NaOH was added and blended at ca. 25 °C for 35 min. The resultant mixture was titrated with 0.5 M HCl (Golachowski, 2003). The percentage of acetylation was calculated according to the method of Wurzburg (1964):

$$\text{percent of acetylation} = \frac{(25 - x) \cdot 0.043 \cdot 0.5 \cdot 100}{a} \left[\text{g}/100\text{g}\right]$$

where x – amount of 0.5 M HCl used for titration of a sample, and a – weight of starch (in conversion to dry weight).

2.4. Determination of the content of adipate groups

One gram of the cross-linked starch was weighed and transferred to a conical flask using 50 mL of distilled water. Then, 1 mL of an aqueous solution of glutaric acid was added to starch, the sample was mixed and 50 mL of 4 N NaOH was added. The sample was mixed for 5 min on a shaker. Afterwards, 20 mL of 12 N HCl were added and the flask was placed in a cold water batch. The cooled mixture was extracted three times with 100 mL of ethyl acetate in a separator into 250 mL. Each time, the upper layer was decanted to a conical flask containing 20 g of anhydrous potassium sulfate. The flask with the mixture was shaken for 10 min in a water bath with a shaker, and then the mixture was filtered. The filtrate was evaporated under vacuum at a temperature of 40 °C, under the pressure of 50 mmHg. Afterwards, 2 mL of pyridine and 1 mL BSTFA were added to dry residues, the mixture was mixed for homogenization and left for 1 h at a room temperature. Then, 2 mL were sampled to an ampoule and analyzed. Chromatographic assays were carried out on a PYE UNICAM series 104 gas chromatograph with an FID detector and a glass column (2.1 m in length and 1.83 mm in diameter). The mobile phase was 5% OV-17 on G DMCS AW chromosorb with 100/120 mesh granulation.

Parameters of separation:

Carrier gas – nitrogen; flow rate – 40 mL/min; detector temperature – 250 C; injector temperature – 240 C; column temperature – 140 C; injection volume – 0.1 μL; retention time: 2.8 min with glutaric acid, 4.5 min with adipic acid.

2.5. Determination of resistance to amyloglucosidase (Zięba et al., 2011a)

Under continuous stirring, starch suspensions were heated to the boiling temperature and then cooled to a temperature of 37 °C at which the samples were hydrolyzed with amyloglucosidase (Amigase by Genecor, Denmark). The concentration of the enzyme was adjusted so as to assure the complete saccharification of pasted native starch after 120 min of the process. The content of free glucose was determined with the use of a CECIL CE 2010 colorimeter (Cecil Instruments, England) at a wavelength of $\lambda = 500$ nm, using a Biosystem company (Spain) reagent for glucose concentration assay that contains glucose oxidase and peroxidase.

2.6. Determination of pasting characteristics with a Brabender viscograph (Zięba et al., 2013)

Pasting characteristics was determined with a Brabender viscograph (Germany), using a measuring vessel (700 cmg type). Briefly, 450 mL of a suspension containing 6 g of starch per 100 g of solution were was prepared in the viscograph vessel. The suspension was heated to a temperature of 40 °C under stirring at 75 rpm and kept at this temperature for 10 min. Afterwards, contents of the vessels were heated at the rate of 1.5 °C/min to a temperature of 94 °C, and kept at this temperature for 10 min. Then, the mixture was cooled to 30 °C at the rate of 1.5 °C/min and kept at this temperature for another 10 min.

2.7. Determination of flow curves of pastes using a Haake oscillating-rotational viscosimeter (Kapelko et al., 2012a)

Suspensions containing 5 g of starch per 100 g of solution were prepared for analyses. They were heated to a tempature of 96 °C for 30 min under continuous stirring. The analysis was carried out with an oscillating – rotational RS 6000 Rheostress viscometer (Haake, Germany). The flow curves of the pastes were determined at a temperature of 50 °C in a system of co-axial cylinders, at a shearing rate of 1–300 s^{-1}. The determined flow curves were described with equations by:

- Oswald de Waele : $\tau = K \cdot \dot{\gamma}^n$
- Casson : $\tau^{0,5} = \tau_{oc}^{0,5} + (\eta_c \cdot \dot{\gamma})^{0,5}$

where τ – shear stress (Pa), K – consistency coefficient (Pa·s^n), $\dot{\gamma}$ – shear rate (s^{-1}), n – flow behavior index, τ_{0C} – yield stress (Pa), and η_C – Casson's plastic viscosity (Pa·s).

2.8. Statistical analysis

The results obtained in the study were analyzed statistically using Statistica 10.0 PL software package. Based on statistical computations (from at least three parallel replications), values of the least significant differences and standard deviations were calculated, and equations of flow curves were determined. For statistical evaluation, the results were subjected to one-way analysis of variance at a significance level of 0.05. Values of the least significant difference (LSD) between the means were computed using the Duncan's test at a significance level of 0.05.

3. Results and discussion

The modified chemical preparations produced from retrograded starch (R-A; R-ADA) were characterized by a higher percentage of esterification than the modified preparations produced under the same conditions from native potato starch (NS-A; NS-ADA) (Table 1). Differences were observed in the degree of substitution with residues of both acetic and adipic acid. Retrograded starch produced through pasting, freezing and defrosting, drying and grinding differs significantly in its form from the native starch occurring in the granular form (Zięba et al., 2011a). Damage of the native structure causes a significant increase in the reactivity of retrograded starch compared to the native starch (Kapelko et al., 2012a; Zięba et al., 2011b).

Table 1
Percentage of esterification of produced modified starch preparations.

Type of preparation	Percentage of esterification with residues of (g/100 g)	
	Acetic acid	Adipic acid
NS-A	2.44 ± 0.01	–
NS-ADA	1.86 ± 0.01	0.13 ± 0.01
R-A	4.29 ± 0.01	–
R-ADA	2.96 ± 0.01	0.23 ± 0.01
LSD0.05	0.31	0.06

Chemical modifications of starch may induce its partial resistance to amylolysis (Leszczyński, 2004; Seung, Kwang, & Hyeon, 2010; Singh et al., 2007). The resistance of acetylated native starch (NS-A) and acetylated distarch adipate (NS-ADA) to the activity of amylases reached ca. 10% (Fig. 2). A similar, relatively low resistance was noted for modified preparations of this type produced under industrial conditions (Le Thanh, Błaszczak, & Lewandowicz, 2007). Significantly higher resistance was determined in the case of retrograded starch esters (R-A 42%; R-ADA 31%). The high amylolytic resistance of retrograded starch acetates has already been reported in our previous works (Kapelko et al., 2012a; Kapelko et al., 2012b; Zięba et al., 2011; Zięba et al., 2011a). The high resistance of such preparations to amylolysis results from high substitution of acetyl residues at the 2nd and 3rd carbon atom that are neighboring the hydrolyzed α-1,4 glycosidic bond (Zięba et al., 2013). A double chemical modification may contribute to the increasing resistance of the resultant preparations (Leszczyński, 2004). A similar dependency was expected to occur in the conducted experiment. It turned out, however, that the R-ADA preparation was characterized by lesser resistance compared to the R-A preparation. This could be due to a relatively low degree of starch esterification with adipic acid and a higher degree of acetylation of the R-A preparation compared to the R-ADA preparation.

After heating, aqueous suspensions of native starch form viscous pastes. Acetylated starch (E1420) and acetylated distarch adipate (E1422) produced under industrial conditions are characterized by increased viscosity of the formed pastes and hence are applied as thickening agents. Acetylated retrograded starch is also capable of forming viscous pastes (Kapelko et al., 2012b; Zięba et al., 2011; Zięba et al., 2013). The pasting characteristics

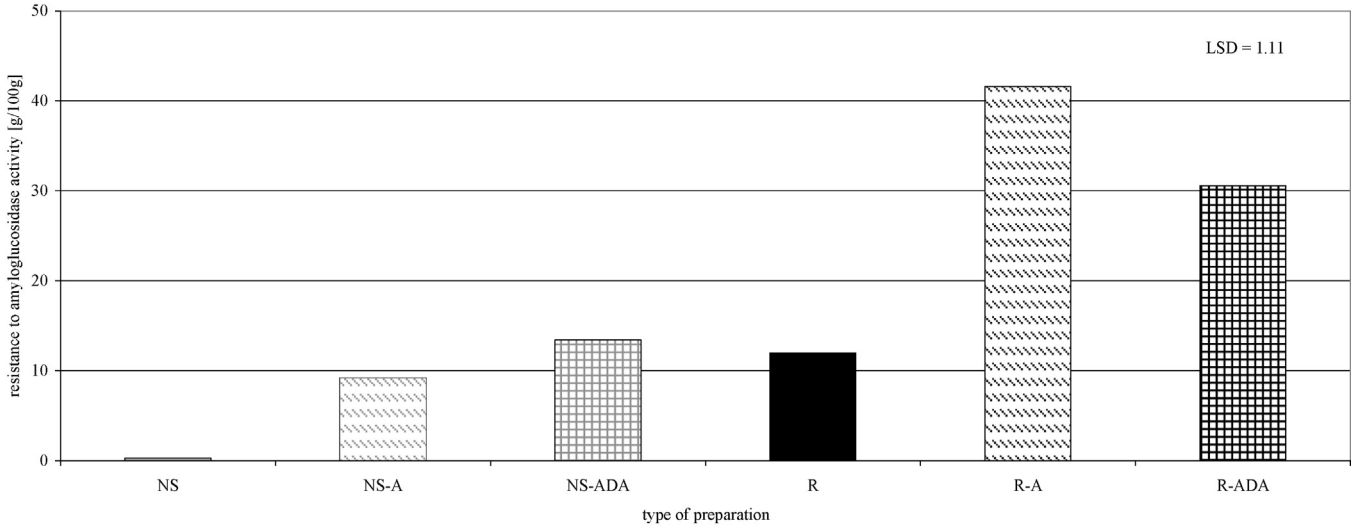


Fig. 2. Resistance of the produced modified starch preparations to the activity of amyloglucosidase.

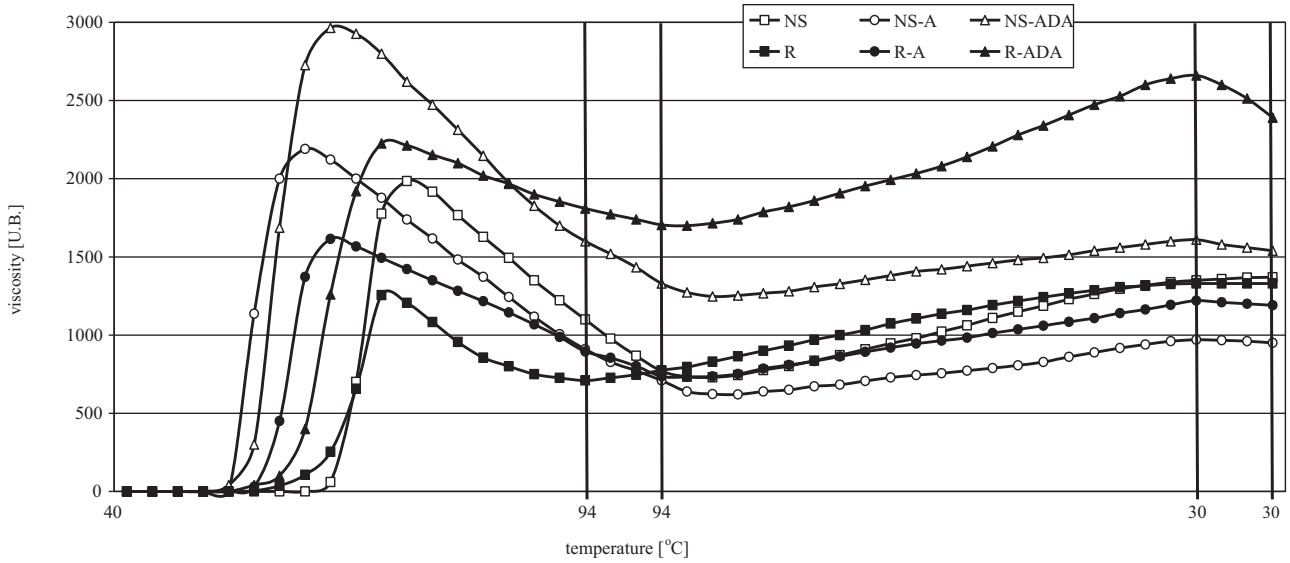
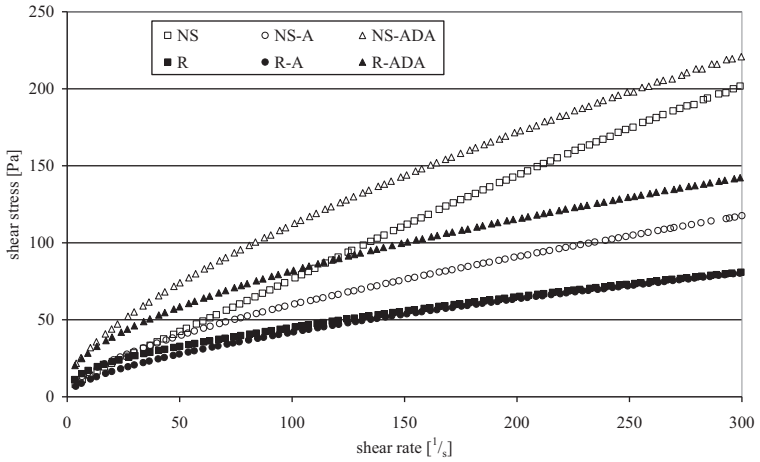


Fig. 3. Pasting characteristics of the produced modified starch preparations.

determined using Brabender viscograph (Fig. 3) and flow curves of the formed pastes (Fig. 4) confirm this property of the modified preparations of retrograded starch. At the initial stage of pasting, viscosity of a 6% paste prepared from native starch reached 1985 B.U. Acetylation increased the maximum viscosity by 10%, whereas acetylation and cross-linking with adipic acid by nearly 50%. The maximum viscosity of the paste produced from retrograded starch was at 1255 B.U. Acetylation increased its maximum viscosity by nearly 30%, whereas cross-linking by 77%. The significant increase in the maximum viscosity of retrograded starch



Type of preparation	model of Ostwald de Waele			model of Casson (n = 0.5)		
	n	K [Pa · s^n]	R^2	τ_{oc} [Pa]	n_c [Pas]	R^2
NS	0.81 ± 0.08	2.14 ± 0.18	0.99	8.91 ± 0.27	0.120 ± 0.002	0.99
NS-A	0.60 ± 0.01	3.99 ± 0.23	0.99	10.45 ± 0.62	0.216 ± 0.011	0.99
NS-ADA	0.61 ± 0.01	6.81 ± 0.40	0.99	17.19 ± 1.04	0.377 ± 0.019	0.99
R	0.49 ± 0.01	4.66 ± 0.10	0.98	11.51 ± 0.24	0.104 ± 0.002	0.99
R-A	0.58 ± 0.01	3.06 ± 0.29	0.98	7.91 ± 0.73	0.139 ± 0.005	0.97
R-ADA	0.49 ± 0.02	8.41 ± 0.65	0.97	20.59 ± 1.47	0.183 ± 0.005	0.98
$LSD_{0.05}$	0.04	0.64	-	1.49	0.03	-

Mean values from three replications ± standard deviations, $LSD_{0.05}$ – least significant differences.

Fig. 4. Flow curves of pastes prepared from modified starch and mathematical parameters describing them.

resulting from its cross-linking caused that the R-ADA preparation was forming pastes with similar viscosity as that of native starch acetate (NS-A). At the second stage of pasting characteristics (during cooling), first a decrease followed by an increase was observed in pastes viscosity. The viscosity of pastes determined at 30 °C ranged from 970 to 1610 B.U. An exception was the paste produced from the R-ADA preparation whose viscosity was considerably higher and reached 2660 B.U. In the entire course of Brabender pasting characteristics, the high viscosity of paste produced from this preparation may be due to relatively high cross-linking (higher than in the case of NS-ADA starch) with residues of adipic acid.

The effect of the conducted modifications on the rheological properties of the produced pastes is confirmed by the plotted flow curves (Fig. 4) and coefficients determined from Oswald de Waele and Casson equations. Good fit of the experimental data to the rheological equations describing them was indicated by high values of R^2 coefficient. All analyzed pastes exhibited non-Newtonian shear-tinned flow with a tendency to yield stress. Values of the coefficient of consistency (K), being a measure of viscosity at the initial stage of shearing, and yield stress (τ_{0C}) were higher in the modified than in the non-modified preparations, except for the R-A preparation. The reason behind relatively low viscosity of the paste made of the R-A preparation may be the high substitution with acetic acid residues, because along with an increase degree of substitution the viscosity of paste prepared from acetate of retrograded starch initially increases and then decreases (Kapelko et al., 2012b). The highest values of these coefficients were determined for the preparations cross – linked with adipic acid (NS-ADA, R-ADA). A similar tendency was observed for Casson's plastic viscosity (η_C) which is a measure of paste resistance to shear forces. The high viscosity of pastes produced from acetylated native starch cross-linked with adipic acid has been described in ample works (Hirsch & Kokini, 2002; Singh et al., 2007; Wattanchant, Muhammad, Hashim, & Rahman, 2003). Very interesting from the practical point of view are rheological properties of the paste produced from the R-ADA preparation, however more explicit research is need to determine the impact of the production method and the degree of cross-linking on properties of such preparations.

4. Conclusion

The conducted modifications of potato starch had a significant impact on the properties of resultant preparation, with direction and extent of these changes depending on the number and type of modifications. Compared to native starch, the retrograded starch (not subjected to chemical modification) was characterized by higher resistance to the activity of amyloglucosidase (12.01 g/100 g), lower viscosity of the prepared paste in the entire course of pasting characteristics, lower values of rheological coefficients determined from the equations describing flow curves, and greater susceptibility of chemical modifications. Acetylation of retrograded starch caused a significant increase (41.61 g/100 g) in resistance to amyloglucosidase activity and relatively small changes in the rheological properties of the prepared pastes compared to the chemically-unmodified preparation. Acetylation of retrograded starch and its cross-linking with adipic acid (compared to the chemically-unmodified preparation) elicited an increase in the resistance to amyloglucosidase action (30.56 g/100 g), viscosity of the prepared paste in the entire course of pasting characteristics, and values of rheological coefficients determined from the equations describing flow curves. The produced preparation of acetylated retrograded starch cross-linked with adipic acid may be deemed an RS3/4 preparation based on its significant resistance to amylolysis. The paste produced from this preparation is characterized by high viscosity and, therefore, may be used as a thickening agent in the food industry.

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