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ORIGINAL ARTICLE

Modification of wheat starch with succinic acid/acetic anhydride and azelaic acid/acetic anhydride mixtures I. Thermophysical and pasting properties

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Abstract The aim of this research was to investigate the influence of modification with succinic acid/acetic anhydride and azelaic acid/acetic anhydride mixtures on thermophysical and pasting properties of wheat starch. Starch was isolated from two wheat varieties and modified with mixtures of succinic acid and acetic anhydride, and azelaic acid and acetic anhydride in 4, 6 and 8 % (w/w). Thermophysical, pasting properties, swelling power, solubility and amylose content of modified starches were determined. The results showed that modifications with mixtures of afore mentioned dicarboxylic acids with acetic anhydride decreased gelatinisation and pasting temperatures. Gelatinisation enthalpy of *Golubica* starch increased, while of *Srpanjka* starch decreased by modifications. Retrogradation after 7 and 14 day-storage at 4 °C decreased after modifications of both starches. Maximum, hot and cold paste viscosity of both starches increased, while stability during shearing at high temperatures decreased. % setback of starches modified with azelaic acid/acetic anhydride mixture decreased. Swelling power and solubility of both starches increased by both modifications.

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Introduction

Wheat starch is widely used in food industry as thickener, stabilizing agent, substitute for flour etc. It is second most produced type of starch in EU (after maize starch) with app. 33 % of total EU starch production (Maningat et al. 2009). Pasting and thermophysical properties are very important for starch application in food industry due to impact of these properties on cost-effectiveness of production, stability of food products and their acceptance by consumers. Native starch, produced by isolation from wheat endosperm, does not meet all demands for its application in food due to constraints in gelatinization, retrogradation and pasting properties, as well as stability at high temperatures and in acidic conditions and therefore has limited application. More often modified starches are used, with properties targetedly changed to endure specific requirements. In a modification process, starch hydrogen bonds are affected in a controllable manner (Balasubramanian et al. 2011).

Esterification of starch is common procedure of chemical modification (Singh et al. 2007). Starch acetates are produced by esterification with acetic anhydride or vinyl acetate (Babić et al. 2007; Gonzales and Perez 2002; Xu et al. 2008) and have lower gelatinization temperatures, higher peak viscosity and reduced retrogradation compared to native counterparts (Wilkins et al. 2003).

Esterification of starch with dicarboxylic acids can result in cross-linking or substitution, since these acids have two carboxylic groups which can react with different starch chains. Modification of starch with mixture of adipic acid and acetic anhydride is commonly applied reaction of starch

cross-linking (Singh et al. 2007; Mali and Grossman 2001). In addition, distarch glutarates (Kim et al. 2008), starch succinates (Lawal 2004) and maleates (Biswas et al. 2006), and starch—dicarboxylic acid complexes (John and Raja 1999) have been prepared. Properties of starches modified with dicarboxylic acids, however, aren't unique, due to possible ways that reaction with starch can take (substitution, cross-linking or combination of both) (Cui 2005).

Succinic acid is dicarboxylic acid used as food additive (E 363) and dietary supplement and is on Food and Drug Administration's (FDA's) list of GRAS ("Generally Recognised as Safe") food additives. In addition to use in food industry, it is also used in pharmaceuticals and cosmetics (Vemuri et al. 2002). Azelaic acid is dicarboxylic acid naturally occurring in wheat, rye and barley (Moniharapon et al. 2005; Sun and Sun 2001; Wu et al. 2001), with reported amount of 4.21–13.76 % in wheat straw extract (Sun and Sun 2001). It is non-teratogenic, non-mutagenic, and lacks acute or chronic toxicity (Moniharapon et al. 2005).

The aim of this research was to investigate the influence of modification with succinic acid/acetic anhydride and azelaic acid/acetic anhydride on thermophysical and pasting properties of wheat starch.

Materials and methods

Starch was isolated from two wheat varieties: *Golubica* and *Srpanjka* as previously described (Ačkar et al. 2010). No proteins were detected in isolated starch, while ash content was 0.29 % in *Golubica* starch and 0.20 % in *Srpanjka*. Complete characterization of starch is shown in: Ačkar et al. (2010).

Modification of starch with succinic acid/acetic anhydride and azelaic acid/acetic anhydride mixtures

Mixture of acetic anhydride and succinic or azelaic acid (30:1) was prepared by suspending:

a) 0.1290 g acid in 3.8710 g acetic anhydride for modification in 4 % (w/w);
b) 0.1935 g acid in 5.8065 g acetic anhydride for modification in 6 % (w/w);
c) 0.2581 g acid in 7.7419 g acetic anhydride for modification in 8 % (w/w)

to achieve 4, 6 or 8 g of modification mixture (containing 1 part of acid and 30 parts of anhydride) per 100 g od dry matter od starch.

Starch (100 g d.m.) was suspended in distilled water (145 mL). Suspension was homogenised by magnetic stirrer (300 rpm/30 min). pH of starch suspension was adjusted to 9.0 with 1 M NaOH and mixture of acid and anhydride was drop-wise added with maintaining pH value close to 9. After addition of mixture of acid and anhydride, starch suspension was stirred for 30 min at room temperature. Overall reaction time was 2 h. Reaction was terminated by adjusting pH to 5.0 with drop-wise addition of 1 M HCl. Suspension was centrifuged (3000 rpm/5 min) and starch pellet was washed with water and centrifuged until supernatant became colorless and no gelatinous lumps on starch pellet were created. Starch suspension was neutralised, centrifuged and starch pellet was air dried. Dry matter content was determined in dried starch by oven drying (130 °C/90 min).

Characterisation of modified starch

Gelatinization and retrogradation properties of native and modified starches were determined by method described by Babić et al. (2009), using differential scanning calorimeter DSC 822^e (Mettler Toledo) equipped with STAR^e software. Starch samples were weighed (18±2 μg) into standard aluminium pan (40 μL) and distilled water was added by Hamilton microsyringe to achieve suspension containing 65 % water. Samples were hermetically sealed and equilibrated for 24 h at room temperature before heat treatment in the DSC apparatus. The starch samples were heated at a rate of 10 °C/min from 25 to 95 °C. After heat treatment, samples were cooled to 25 °C and removed from DSC. The starch gels were aged at 4 °C and monitored for retrogradation after 7 and 14 days. The retrogradation experiments were conducted at a heating rate of 10 °C/min from 25 to 95 °C. The changes in enthalpy (ΔH in J/g of dry starch), onset temperature (T_o), peak temperature (T_p) and conclusion temperature (T_c) for gelatinisation and retrogradation were obtained from the exotherm DSC curves. Experiments were run in triplicates.

Pasting properties of starches (7 % d.w.b., 100 g total weight) were determined using a micro visco-amylograph (Model 803202, Brabender Gmbh & Co KG, Duisburg, Germany). The starch suspensions were heated at 7.5 °C/min from 30 °C to 92 °C, held at 92 °C for 20 min, cooled at 7.5 °C/min to 50 °C, and held at 50 °C for 20 min. Experiments were run in triplicates.

Swelling power (SP) and solubility (SOL) were determined by method described by Babić et al. (2007). 1 % starch suspensions (d.w.b.) were heated in shaking water bath for 30 min at 65, 75, 85 and 95 °C. This temperature range is commonly used in SP and SOL measurements. After heating suspensions were cooled and centrifuged at 4000 rpm for 30 min. Supernatant was decanted, and gel was weighed. Dry matter of supernatant was determined by oven drying at 105 °C

until constant mass was reached. Experiments were run in triplicates. SP and SOL were calculated from Eqs. 1. and 2.:

$$SP = weight\ of\ gel / weight\ of\ dry\ matter\ in\ gel\ [g/g] \quad (1)$$

$$SOL = weight\ of\ dry\ matter\ in\ supernatant / weight\ of\ dry\ matter\ in\ suspension\ [\%] \quad (2)$$

Amylose content was determined according to the Megazyme method K-AMYL 04/06. Starch samples were completely dispersed in dimethyl sulphoxide (DMSO), precipitated in ethanol and dissolved in acetate/salt solution. Amylopectin was specifically precipitated by the addition of ConA and removed by centrifugation. The amylose in the aliquot of the supernatant was enzymatically hydrolysed to D-glucose, which was analysed using glucose oxidase/peroxidase reagent. Total starch in separate aliquot of acetate/salt solution was also enzymatically hydrolysed to nglucose, which was analysed using glucose oxidase/peroxidase reagent. Concentration of amylose in starch sample was calculated from Eq. 3.:

$$Amylose = [Absorbance(ConA)/Absorbance(Total\ Starch\ Aliquot)] \times 66.8\ [\%] \quad (3)$$

Where 66.8 was dilution factor for ConA and Total Starch extracts.

All experimental data were analysed by analysis of variance (ANOVA) and Fisher's least significant difference (LSD) with significance defined at $P<0.05$. All statistical analyses were carried out using software program STATISTICA 8 (StatSoft, Inc, USA), on three replicates.

Results and discussion

Properties of native *Golubica* and *Srpanjka* starches are previously described (Ačkar et al. 2010).

DSC gelatinisation properties of starch isolated from wheat varieties *Golubica* (G) and *Srpanjka* (S) and modified with succinic acid/acetic anhydride (SA) and azelaic acid/acetic anhydride (AZA) mixtures in 4, 6 and 8 % are shown in Table 1. Gelatinisation is process which requires heating starch in significant content of water. It includes granule swelling, amylose leaching, formation of gel- or paste-like mass and amylopectin fusion (Brouillet-Fourman et al. 2003). Not all starch granules begin to gelatinise at exact same temperature and therefore gelatinisation temperature is defined as relatively narrow temperature range rather than one specific temperature (Thomas and Atwell 1999). Onset, peak and endset gelatinisation temperatures decreased by modification of both starches with investigated mixtures of dicarboxylic acids and acetic anhydride. In addition, temperature range of gelatinisation (Δt) increased. These phenomena indicated that modification had influenced internal crystalline structure of starch granules, changing crystal shapes and sizes, degree of crystal perfection and type of chain intertwining (linear-linear, linear-branch, branch-branch) making it more easily destroyed at lower temperature than native starch (Kim et al. 2008). Liu et al. (1999) observed that cross-linked normal rice starch had lower gelatinisation temperature than native counterpart, while cross-linked waxy starch had higher onset gelatinisation temperature. Both normal and waxy acetylated starches (in the same research) had lower gelatinisation temperature. In addition, adley starch modified with glutaric acid had lower gelatinisation temperatures and broader temperature range than native counterpart (Kim et

Table 1 Gelatinisation properties of starch isolated from wheat varieties *Golubica* (G) and *Srpanjka* (S) and modified with succinic acid/acetic anhydride (SA) and azelaic acid/acetic anhydride (AZA) mixtures in 4, 6 and 8 %

	t_o/°C	t_p/°C	t_e/°C	Δt/°C	ΔH/J/g
G	59.4±0.07^{d}	62.6±0.03^{d}	66.6±0.06^{d}	7.2	7.1±0.10^{a}
GSA4	57.6±0.20^{c}	61.5±0.09^{c}	65.7±0.22^{c}	8.1	8.1±0.04^{c}
GSA6	55.8±0.05^{a}	59.3±0.12^{a}	63.4±0.19^{a}	7.6	7.6±0.17^{b}
GSA8	56.9±0.10^{b}	60.4±0.09^{b}	64.7±0.12^{b}	7.8	7.8±0.12^{b}
GAZA4	57.9±0.05^{c}	61.8±0.05^{c}	65.9±0.10^{c}	8.0	7.9±0.11^{c}
GAZA6	56.1±0.15^{a}	59.7±0.10^{a}	63.8±0.17^{a}	7.7	7.6±0.21^{b}
GAZA8	56.6±0.09^{b}	60.3±0.10^{b}	64.4±0.06^{b}	7.8	7.8±0.12^{b,c}
S	59.9±0.17^{d}	63.7±0.02^{d}	67.8±0.09^{d}	7.9	8.4±0.01^{d}
SSA4	59.0±0.07^{c}	63.0±0.13^{c}	67.4±0.12^{c}	8.4	8.2±0.05^{c}
SSA6	56.8±0.13^{b}	61.5±0.13^{b}	65.9±0.09^{b}	9.1	7.7±0.05^{b}
SSA8	55.2±0.05^{a}	59.7±0.09^{a}	64.2±0.15^{a}	9.0	7.4±0.02^{a}
SAZA4	58.4±0.44^{c}	62.2±0.12^{b}	66.3±0.06^{c}	7.9	7.4±0.04^{c}
SAZA6	57.2±0.06^{b}	62.1±0.08^{b}	63.8±0.17^{a}	6.6	7.1±0.01^{b}
SAZA8	55.4±0.06^{a}	60.6±0.35^{a}	64.8±0.07^{b}	9.4	6.6±0.01^{a}

t_o, onset temperature; t_p, peak temperature; t_e, endset temperature; $\Delta t = t_o - t_e$, *temperature range of gelatinisation* ΔH, gelatinisation entalphy; Values are means ± SD of triplicate. Values in the same column with different superscripts (a-d) are significantly different than native counterparts ($p<0.05$)

al. 2008). Both hydroxypropylated and dual-modified wheat starches had lower gelatinisation temperatures than native starch (Van Hung and Morita 2005). In *Golubica* starch, slight increase of onset and peak gelatinisation temperature was observed when reagent mixture content was increased from 6 % to 8 %, probably due to more pronounced effect of cross-linking. Gelatinisation enthalpy of *Golubica* starch increased, while gelatinisation enthalpy of *Srpanjka* starch decreased by both investigated modifications in all proportions. Gelatinisation enthalpy is measure of crystallinity (quantity and quality) and is indicator of loss of molecular order within granule on gelatinisation (Singh et al. 2007). Decrease of gelatinisation enthalpy can be result of reduced proportion of starch that can be gelatinised due to cross-linking at lower levels, or disruption of double helices within amorphous regions of the granules due to substitution (Singh et al. 2007). Kim et al. (2008) observed lower gelatinisation enthalpy of glutarate starches compared to native counterpart, ascribing this phenomenon to disruption of molecular and crystalline order. Increase of gelatinisation enthalpy can be result of increase of proportion of starch granules that gelatinise due to weakening inter- and intramolecular bonds by introduction of substituent groups, or caused by higher amount of energy required for disruption of highly ordered crystallites formed by cross-linking at higher level (Singh et al. 2007).

Upon cooling, less energy is available to keep solubilised starch molecules apart and starch fragments re-associate. Starch chains form a simple juncture point which then may develop into more extensively ordered regions and, ultimately, crystalline form appears (Thomas and Atwell 1999). This process is called retrogradation and is most extensive at 4 °C. Since retrogradation has negative impact on quality and acceptability of starch based products (Schiraldi et al. 1996; Kingcam et al. 2008), retardation/decrease of retrogradation is preferable. Retrogradation enthalpies of *Golubica* and *Srpanjka* starch modified with both succinic acid/acetic anhydride and azelaic acid/acetic anhydride mixtures in all investigated concentrations were decreased after 7 and 14 days of storage at 4 °C (Table 2 and 3), with more pronounced effect of modification with mixture of succinic acid and acetic anhydride. Decrease of retrogradation enthalpy indicated that fewer crystals had been formed during storage and therefore directly showed decrease of retrogradation. In addition, lower onset, peak and endset temperatures observed during measurement of retrogradation showed that retrogradation resulted in crystalline forms that were different in nature from those present in ungelatinised starch (Karim et al. 2000).

Pasting properties of starch are another phenomena crucial for starch application in food. Pasting process proceeds gelatinisation. With excess heat, more granules become swollen, viscosity increases and peak viscosity is reached when maximum number of swollen intact granules is present. Continued heating eventually results in decrease of viscosity of most starches due to disruption of granules and leaching polymers (Thomas and Atwell 1999). Decrease of viscosity is proportional to break down of granules and

Table 2 Retrogradation properties of starch isolated from wheat varieties *Golubica* (G) and *Srpanjka* (S) and modified with succinic acid/acetic anhydride (SA) and azelaic acid/acetic anhydride (AZA) mixtures in 4, 6 and 8 % after 7 days of storage at 4 °C

	t_o/°C	t_p/°C	t_e/°C	ΔH/J/g
G	42.5±0.29^{a,b}	52.1±0.26^{b}	60.4±0.05^{a}	3.4±0.01^{c}
GSA4	42.8±0.26^{b}	51.9±0.15^{b}	60.3±0.21^{a}	2.3±0.03^{b}
GSA6	42.4±0.47^{a,b}	51.1±0.36^{a}	60.3±0.50^{a}	2.3±0.02^{b}
GSA8	42.0±0.15^{a}	51.0±0.36^{a}	60.2±0.55^{a}	2.1±0.02^{a}
GAZA4	42.4±0.29^{a,b}	52.2±0.06^{b}	60.2±0.10^{a}	2.5±0.01^{c}
GAZA6	42.1±0.35^{a}	51.2±0.38^{a}	60.2±0.12^{a}	2.4±0.01^{a}
GAZA8	42.7±0.06^{b}	51.3±0.18^{a}	60.2±0.25^{a}	2.4±0.02^{b}
S	43.6±0.42^{c}	51.6±0.27^{a,b}	60.4±0.49^{b}	3.2±0.03^{b}
SSA4	41.9±0.23^{a}	51.2±0.25^{a}	59.8±0.15^{a}	2.1±0.07^{a}
SSA6	42.5±0.31^{b}	51.6±0.26^{a,b}	60.6±0.49^{b}	2.1±0.05^{a}
SSA8	42.4±0.15^{a,b}	52.0±0.40^{b}	60.7±0.14^{b}	2.1±0.07^{a}
SAZA4	42.7±0.25^{b}	51.8±0.23^{b}	59.8±0.10^{a}	2.3±0.03^{b}
SAZA6	41.8±0.15^{a}	50.9±0.23^{a}	59.9±0.15^{a,b}	2.2±0.03^{a}
SAZA8	42.1±0.20^{a}	51.0±0.33^{a}	60.2±0.29^{a,b}	2.2±0.02^{a}

t_o, onset temperature; t_p, peak temperature; t_e, endset temperature; ΔH, retrogradation entalphy; Values are means ± SD of triplicate. Values in the same column with different superscripts (a-d) are significantly different than native counterparts ($p<0.05$)

Table 3 Retrogradation properties of starch isolated from wheat varieties *Golubica* (G) and *Srpanjka* (S) and modified with succinic acid/acetic anhydride (SA) and azelaic acid/acetic anhydride (AZA) mixtures in 4, 6 and 8 % after 14 days of storage at 4 °C

	t_o/°C	t_p/°C	t_e/°C	ΔH/J/g
G	42.5±0.29^{a,b}	52.1±0.26^{b}	60.4±0.05^{a}	3.4±0.01^{c}
GSA4	42.8±0.26^{b}	51.9±0.15^{b}	60.3±0.21^{a}	2.3±0.03^{b}
GSA6	42.4±0.47^{a,b}	51.1±0.36^{a}	60.3±0.50^{a}	2.3±0.02^{b}
GSA8	42.0±0.15^{a}	51.0±0.36^{a}	60.2±0.55^{a}	2.1±0.02^{a}
GAZA4	42.1±0.44^{a}	52.6±0.35^{b}	60.6±0.44^{a}	3.2±0.02^{c}
GAZA6	41.8±0.15^{a}	51.2±0.40^{a}	60.4±0.15^{a}	2.9±0.07^{b}
GAZA8	42.1±0.15^{a}	51.4±0.10^{a}	61.0±0.31^{a}	2.8±0.01^{a}
S	43.1±0.32^{b,c}	51.5±0.24^{a}	60.6±0.06^{b}	3.7±0.04^{c}
SSA4	43.3±0.21^{c}	53.2±0.40^{b}	61.3±0.38^{c}	2.4±0.05^{b}
SSA6	41.7±0.10^{a}	53.4±0.21^{b}	60.3±0.05^{a,b}	2.4±0.05^{a}
SSA8	42.8±0.38^{b}	51.3±0.46^{a}	60.1±0.32^{a}	2.3±0.06^{a}
SAZA4	43.3±0.12^{a}	53.4±0.18^{b}	60.8±0.09^{a}	2.4±0.05^{b}
SAZA6	44.7±0.25^{b}	54.6±0.21^{c}	61.1±0.28^{a}	2.4±0.02^{b}
SAZA8	43.5±0.21^{a}	53.5±0.30^{b}	60.6±0.47^{a}	2.0±0.04^{a}

t_o, onset temperature; t_p, peak temperature; t_e, endset temperature; ΔH, retrogradation entalphy; Values are means ± SD of triplicate. Values in the same column with different superscripts (a-d) are significantly different than native counterparts ($p<0.05$)

Table 4 Pasting properties of starch isolated from wheat varieties *Golubica* (G) and *Srpanjka* (S) and modified with succinic acid/acetic anhydride (SA) and azelaic acid/acetic anhydride (AZA) mixtures in 4, 6 and 8 %. Pastes contained 7 % of starch (w/w)

Starch	Pasting Temp./°C	Peak viscosity/BU	Viscosity at 92 °C/BU	After 20 min at 92 °C/BU	Viscosity at 50 °C/BU	After 20 min at 50 °C/BU	Breakdown*	Breakdown/% (peak v.)**	Setback*	Setback/% (v. at 50 °C)**
G	64.8±0.25^{c}	306.3±5.51^{a}	256.6±3.79^{a}	240.6±4.16^{a}	501.6±4.04^{a,b}	435.0±4.58^{c}	65.6±2.08^{a}	21.4	261.0±1.73^{a}	108.4
GSA4	62.6±0.32^{b}	368.6±3.06^{b}	362.0±3.61^{b}	224.6±3.06^{a}	507.6±5.77^{b}	412.6±5.51^{b}	144.0±0.00^{b}	39.0	283.0±4.00^{c}	125.9
GSA6	60.1±0.07^{a}	447.0±4.24^{c}	438.5±4.95^{c}	254.0±4.24^{c}	512.0±5.66^{b}	437.5±4.95^{c}	193.0±0.00^{d}	43.1	258.0±1.41^{a}	101.5
GSA8	62.2±0.14^{b}	373.5±3.54^{b}	369.5±4.95^{b}	225.0±0.00^{a}	496.0±5.66^{a}	388.5±4.95^{a}	148.5±3.54^{c}	39.7	271.0±5.66^{b}	120.4
GAZA4	62.6±0.07^{c}	473.0±1.41^{c}	457.0±11.31^{b}	382.0±8.49^{c}	762.5±7.78^{c}	595.0±7.07^{b}	91.0±7.07^{c}	19.2	380.5±0.71^{c}	99.6
GAZA6	61.1±0.42^{a}	519.5±2.12^{d}	519.0±1.41^{c}	366.5±3.54^{b}	692.5±4.95^{b}	588.5±6.36^{b}	153.0±5.66^{d}	29.4	326.0±8.49^{b}	88.9
GAZA8	61.8±0.07^{b}	462.5±3.54^{b}	458.5±0.71^{b}	417.0±4.24^{d}	847.0±9.90^{d}	643.0±4.24^{c}	45.5±7.78^{a}	9.8	430.0±14.14^{d}	103.1
S	65.6±0.36^{d}	291.0±3.61^{a}	272.3±4.16^{a}	214.0±3.00^{b}	457.6±3.79^{a}	391.6±4.04^{b,c}	77.0±2.65^{a}	26.4	243.6±1.53^{a}	113.8
SSA4	63.7±0.28^{c}	329.0±1.41^{b}	328.0±0.00^{b}	205.0±2.83^{a}	455.0±2.83^{a}	366.5±4.95^{a}	124.0±1.41^{b}	37.6	250.0±0.00^{a}	121.9
SSA6	62.0±0.28^{b}	380.0±2.83^{c}	376.0±1.41^{c}	229.5±2.12^{c}	490.0±2.83^{b}	382.0±5.66^{b}	150.5±4.95^{c}	39.6	260.5±0.71^{b}	113.5
SSA8	60.5±0.14^{a}	413.0±4.24^{d}	406.0±5.66^{d}	240.5±3.54^{d}	490.0±2.83^{b}	400.0±0.00^{c}	172.5±0.71^{d}	41.7	249.5±6.36^{a}	103.7
SAZA4	63.1±0.21^{b}	436.5±6.36^{b}	431.5±3.54^{b}	318.0±2.83^{c}	637.5±4.95^{b}	493.0±7.07^{c}	118.5±3.54^{c}	27.1	319.5±2.12^{b}	100.4
SAZA6	62.5±0.14^{b}	459.5±4.95^{c}	451.5±3.54^{c}	402.5±2.12^{d}	758.5±4.95^{c}	582.0±5.66^{d}	57.0±2.83^{a}	12.4	356.0±2.83^{d}	88.4
SAZA8	61.1±0.35^{a}	444.0±1.41^{b}	440.5±3.54^{b}	303.5±0.71^{b}	632.0±5.66^{b}	469.5±6.36^{b}	140.5±2.12^{d}	31.6	328.5±4.95^{c}	108.2

Values are means ± SD of triplicate. Values in the same column with different superscripts (a-d) are significantly different than native counterparts ($p<0.05$)

*Breakdown = Peak viscosity-viscosity at 92 °C after 20 min; Setback = viscosity at 92 °C after 20 min—viscosity at 50 °C before holding

**Breakdown/%(peak v.) = (breakdown × 100)/peak viscosity; Setback/% (v. at 50 °C) = (setback × 100)/viscosity at 50 °C

loss of structural integrity. Pasting properties of *Golubica* and *Srpanjka* starch modified with investigated mixtures of dicarboxylic acids and acetic anhydride are shown in Table 4. Pasting temperature of both starches decreased after modification with succinic acid/acetic anhydride and azelaic acid/acetic anhydride in 4, 6 and 8 %, with proportional correlation for *Srpanjka* starch. Singh et al. (2006) observed same phenomenon for acetylated rice starches. However, Luo et al. (2009) determined increase of pasting temperature of waxy potato starch modified with mixture of adipic acid and acetic anhydride. Maximum viscosity, viscosity at 92 °C and 50 °C of both investigated starches increased by both modifications, with more significant influence of azelaic acid/acetic anhydride mixtures. Hot paste viscosity also increased by modification of waxy potato starch with adipic acid/acetic anhydride mixture (Luo et al. 2009). Hui et al. (2009) stated that introduction of bulky octenylsuccinic group into starch structure enhanced its pasting capacity. However, Bhandari and Singhal (2002) reported increase of peak viscosity and decrease of hot and cold paste viscosity after succinylation of corn and amaranth starches, while Lawal (2004) found that succinylation and acetylation of maize starch had resulted in decrease of peak, hot and cold paste viscosity.

Stability of starch pastes during shearing at high temperatures (breakdown value) and upon cooling (setback) is shown in Table 4. Breakdown value is measure of fragility of granules and their resistance to disintegration due to heating and shearing (Hernandez-Uribe et al. 2004). From increased breakdown values, it can be observed that both modifications decreased stability of starch paste, except *Golubica* starch modified with azelaic acid/acetic anhydride mixture in 4 and 8 % and *Srpanjka* starch modified with azelaic acid/acetic anhydride mixture in 6 %. Adebowale and Lawal (2003) stated that starches that are capable to swell to higher degree are also less resistant to breakdown and show significant viscosity decrease after maximum viscosity is reached. Results of this research are in consistence with this observation.

According to Adebowale et al. (2002), setback reflects tendency of starch paste towards gel formation. Highest increase of viscosity upon cooling was observed for *Golubica* starch modified with succinic acid/acetic

Table 5 Swelling power of starch isolated from wheat varieties *Golubica* (G) and *Srpanjka* (S) and modified with succinic acid/acetanhydride (SA) and azelaic acid/acetanhydride (AZA) mixtures in 4, 6 and 8 %

Starch	65 °C	75 °C	85 °C	95 °C
G	8.6±0.50^{a}	9.1±0.19^{a}	11.4±0.04^{a}	22.2±0.20^{a}
GSA4	9.2±0.06^{a}	11.6±0.27^{b}	16.5±0.60^{b}	27.8±0.21^{b}
GSA6	11.4±0.30^{c}	15.5±0.22^{d}	34.4±0.94^{d}	42.9±0.73^{c}
GSA8	10.4±0.15^{b}	13.2±0.33^{c}	21.2±0.27^{c}	51.3±0.34^{d}
GAZA4	8.8±0.03^{a,b}	10.6±0.20^{b}	14.9±0.15^{b}	24.2±0.43^{b}
GAZA6	10.6±0.25^{c}	13.9±0.04^{d}	25.5±0.78^{d}	27.7±0.05^{c}
GAZA8	9.4±0.12^{b}	11.9±0.04^{c}	17.7±0.32^{c}	27.0±0.59^{c}
S	8.2±0.12^{a}	9.3±0.02^{a}	11.9±0.17^{a}	23.2±0.65^{a}
SSA4	9.2±0.01^{b}	11.8±0.10^{b}	17.8±0.02^{b}	36.0±0.94^{b}
SSA6	10.1±0.05^{c}	13.1±0.23^{c}	19.8±0.03^{c}	42.3±0.53^{c}
SSA8	11.2±0.16^{d}	15.2±0.19^{d}	24.8±0.54^{d}	57.1±0.98^{d}
SAZA4	10.5±0.05^{d}	11.7±0.01^{b}	18.4±0.50^{b}	30.5±0.76^{c}
SAZA6	9.8±0.00^{b}	12.3±0.07^{c}	17.7±0.06^{b}	28.6±0.30^{b}
SAZA8	10.2±0.02^{c}	13.2±0.09^{d}	20.8±0.25^{c}	32.8±0.77^{d}

Values are means ± SD of triplicate. Values in the same column with different superscripts (a-d) are significantly different than native counterparts ($p<0.05$)

Table 6 Solubility of starch isolated from wheat varieties *Golubica* (G) and *Srpanjka* (S) and modified with succinic acid/acetic anhydride (SA) and azelaic acid/acetic anhydride (AZA) mixtures in 4, 6 and 8 %

Starch	65 °C	75 °C	85 °C	95 °C
G	3.8±0.22^{a}	6.0±0.82^{a}	11.6±0.02^{a}	32.6±1.03^{a}
GSA4	8.9±0.24^{b}	14.5±1.19^{b}	21.5±2.52^{b}	35.5±1.48^{a}
GSA6	11.2±0.00^{c}	17.2±0.08^{c}	35.5±1.12^{d}	41.5±2.05^{b}
GSA8	12.1±0.24^{d}	15.9±0.00^{b,c}	28.1±0.08^{c}	46.0±0.08^{c}
GAZA4	8.1±0.25^{b}	11.4±0.05^{b}	19.0±0.13^{b}	31.6±0.37^{b}
GAZA6	8.7±0.18^{b}	14.0±0.29^{c}	29.3±0.15^{d}	31.8±0.15^{b}
GAZA8	10.0±0.24^{c}	12.8±0.16^{c}	22.0±0.26^{c}	29.6±0.21^{a}
S	4.5±0.30^{a}	6.8±0.42^{a}	13.3±0.04^{a}	35.8±0.24^{a}
SSA4	9.9±0.08^{b}	15.0±0.12^{b}	26.7±0.27^{b}	44.5±0.71^{b}
SSA6	11.4±0.50^{c}	16.9±0.23^{c}	28.0±0.78^{c}	45.1±0.74^{b}
SSA8	12.2±0.20^{c}	18.1±0.45^{d}	29.4±0.37^{d}	47.0±0.55^{c}
SAZA4	8.5±0.19^{b}	13.1±0.17^{b}	23.0±0.02^{b}	35.3±0.16^{b}
SAZA6	10.9±0.17^{c}	13.1±0.03^{b}	23.3±0.05^{b}	31.3±0.19^{a}
SAZA8	11.0±0.29^{c}	16.2±0.29^{c}	26.8±0.32^{c}	36.1±0.07^{c}

Values are means ± SD of triplicate. Values in the same column with different superscripts (a-d) are significantly different than native counterparts ($p<0.05$)

Table 7 Amylose content in starch isolated from wheat varieties *Golubica* (G) and *Srpanjka* (S) and modified with succinic acid/acetic anhydride (SA) and azelaic acid/acetic anhydride (AZA) mixtures in 4, 6 and 8 %

% reagens used	% amylose			
	GSA	GAZA	SSA	SAZA
0 (native)	20.2±0.32^{b}	20.2±0.32^{a}	22.4±2.01^{c}	22.4±2.01^{b}
4	24.0±1.12^{c}	27.1±0.66^{c}	22.0±0.52^{c}	10.5±0.43^{a}
6	16.8±0.76^{a}	25.7±0.46^{b}	16.7±0.18^{b}	8.9±0.83^{a}
8	18.5±0.16^{a,b}	20.3±0.11^{a}	13.0±1.41^{a}	11.1±0.02^{a}

Values are means ± SD of triplicate. Values in the same column with different superscripts (a-d) are significantly different than native counterparts ($p<0.05$)

anhydride mixture in 4 % (125.96 % increase), while *Srpanjka* starch had lowest change of viscosity (88.44 % increase) (Table 5). In addition, modification with succinic acid/acetic anhydride increased, while azelaic acid/acetic anhydride decreased %setback values of both starches. Lower setback values are result of restriction of realignment of starch molecules due to steric hindrance of substituent groups, while high cold paste viscosity is characteristic of cross-linked starches (Singh et al. 2007).

Swelling power indicates water holding capacity of starch, which is important for food quality and texture (Kaur et al. 2011). Swelling power of starch isolated from wheat varieties *Golubica* (G) and *Srpanjka* (S) and modified with succinic acid/acetic anhydride (SA) and azelaic acid/acetic anhydride (AZA) mixtures in 4, 6 and 8 % is shown in Table 5. Swelling power of all investigated starches increased proportionally to temperature increase. This is due to thermodynamic activation of starch molecules and their increased mobility which enhance water penetration (Lawal 2004). In addition, modifications resulted in increase of swelling power, but not proportionally to degree of modification. Bulky ester groups cause steric hindrance and repulsion between starch molecules which facilitates water percolation within amorphous regions of granules (Lawal 2004). However, cross-linking enhances bonding between starch chains and reduces swelling power. Since with increase of modification degree concentration of dicarboxylic acids which can cross-link starch increases, non-linear increase of swelling power may be due to introduction of more cross-linking bonds in addition to substituent acetyl groups. Lawal (2004) reported increase of swelling power due to acetylation and succinylation of maize starch, and Raina et al. (2006) determined the same phenomenon for dual-modified rice starch. However, swelling power of waxy potato starch decreased after modification with adipic acid/acetic anhydride mixture (Luo et al. 2009), while swelling power of adley starch glutarate depended on degree of modification (Kim et al. 2008).

Increase of solubility due to modification with investigated dicarboxylic acid/acetic anhydride mixtures (Table 6) was more significant than increase of swelling power both for *Golubica* and *Srpanjka* starch. While swelling power is property of amylopectin, solubility is mainly result of amylose leaching from starch granule (Eliasson and Gudmundsson 2006). Higher values of solubility may be result of starch morphology changes which occur during chemical modifications (Singh et al. 2007). However, correlation between amylose content and solubility wasn't clearly established in this research. Amylose content of modified *Srpanjka* starch (Table 7) decreased, and same trend was observed for wheat starch modified with HCl (Kaur et al. 2007). However, amylose content of *Golubica* starch increased by modification with succinic acid/acetic anhydride mixture in 4 % and azelaic acid/acetic anhydride mixture in 4 and 6 %. This phenomenon is yet to be explained—whether linearization of amylopectin occurred, or chemical modification caused inhibition of enzymes used in the assay.

Conclusions

From results shown, it is visible that mixtures of succinic or azelaic acid with acetic anhydride can be used for production of starches with decreased temperature of gelatinisation and tendency towards retrogradation, and increased swelling power and solubility. Modification with succinic acid/acetic anhydride increased, while azelaic acid/acetic anhydride decreased %setback values, which shows potentiality of succinic acid/acetic anhydride mixture application in production of starches with high tendency for gel formation.

Wheat starch modified with succinic acid or azelaic acid/acetic anhydride mixture has potential application in systems where high stability during storage (low retrogradation) is required along with high paste viscosity.

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