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Isolation of starch from two wheat varieties and their modification with epichlorohydrin

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ABSTRACT

The aim of this research was determination of influence of wheat variety and modification with epichlorohydrin on starch properties. Starch was isolated from two wheat varieties: "Golubica" and "Srpanjka" and modified with different concentrations of epichlorohydrin (0.1%, 0.3% and 0.5% v/w). Both native and modified starches were characterised. Results showed similar characteristics of native wheat starches. Modification with epichlorohydrin increased temperature of gelatinisation, but gelatinisation and retrogradation enthalpy were affected by the extent of the chemical reaction between starch and epichlorohydrin, due to differences in reactivity between wheat starch varieties. Maximum viscosity decreased as well as breakdown and setback values. Swelling power and solubility were also decreased by modification, while paste clarity and freeze–thaw stability were influenced differently, due to different extent of the chemical reaction between different starch varieties and epichlorohydrin. Colour of starch was not significantly altered by modification. Digestibility of starches can be reduced by proper selection of epichlorohydrin concentration used for modification.

1. Introduction

Wheat is one of the most common and most grown cultivars used in human diet. In addition to its use in production of flour, bread and pasta, it is widely used in production of starch. Starch is used in numerous food industry applications, such as thickening, gelling, stabilising, fat replacement, etc. In some cases, native starch does not meet the functional properties required in food products. Therefore, starches used in food industry are often modified to overcome undesirable changes in product texture and appearance caused by retrogradation or breakdown of starch during processing and storage (Babić et al., 2006).

Cross-linked starches constitute major class of modified starches. Cross-linking via bi- or polyfunctional reagents reinforces already present hydrogen bonds in granules with new covalent bonds. As a result, cross-linked starch is more resistant to acid, heat and shearing than native counterpart (Jyothi, Moorthy, & Rajasekharan, 2006; Kaur, Singh, & Singh, 2006).

The most widely used cross-linking reagents include adipic and acetic mixed anhydrides, phosphorus oxychloride, sodium trimetaphosphate and epichlorohydrin (EPI). Cross-linking of starch with EPI is the most common method used in polysaccharide chemistry. Reaction of starch with EPI gives distarch glycerols, which are highly resistant to pH and mechanical shear due to formation of ether linkages between the cross-links and the hydroxyl groups in the starch (Jyothi et al., 2006).

The aim of this research was to determine influence of wheat varieties and modification with epichlorohydrin on starch properties. Starch was isolated from Croatian wheat varieties "Golubica" and "Srpanjka", modified with epichlorohydrin in 0.1%, 0.3% and 0.5% v/w concentrations, and native and modified starches were characterised.

2. Materials and methods

Wheat varieties "Golubica" and "Srpanjka" (harvest 2008) were obtained from Agricultural Institute, Osijek, Croatia. According to the data provided with samples, "Golubica" variety contained 67.00% d.m. starch, 14.37% d.m. protein and 12.27% moisture, while "Srpanjka" variety contained 68.73% d.m. starch, 12.57% d.m. protein and 12.20% moisture. Epichloroydrin was product of Fluka, Germany. All chemicals used in the experiment were of analytical grade.

2.1. Isolation of starch from wheat

Starch was isolated from wheat by following procedure: Wheat was fully milled in laboratory miller (IKA Werke M20), sifted and flour was suspended in 0.25% NaOH (1:3 w/w) by stirring with

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propeller stirrer (Tehtnica UM-405) at 1500 rpm for 1 h at room temperature. Suspension was centrifuged at 3000 rpm for 5 min in benchtop centrifuge IEC Centra MP4R and upper greyish layer was discarded. Sediment containing starch was washed with distilled water over cheese cloth and filter cloth. Washings were centrifuged (3000 rpm for 5 min) and upper grey layer of proteins was removed with spatula. White starch sediment was suspended in water and centrifugation and re-suspension of starch in water were repeated until grey layer disappeared. Washed starch was neutralised with 1 M HCl, centrifuged once more and air-dried. Dry matter content of starch was determined by oven drying (130 °C/90 min).

2.2. Modification of starch with epichlorohydrin

Starch was modified by method followed by Wurtzburg (1960). 100 g of wheat starch (d.w.b.) was suspended in 150 ml of 0.5% NaOH. Epichlorohydrin (0.1%, 0.3% or 0.5% v/w, d.w.b. respectively) was slowly added with agitating at magnetic agitator. After agitating for 5 h at room temperature, reaction was terminated by adjustment of pH of suspension to 5.0 with 1 M HCl. Starch was centrifuged (3000 rpm for 5 min) and washed with distilled water. Washing with water and centrifugation steps were repeated until no gelatinous lumps were formed on starch surface. Thereupon, starch was neutralised with 1 M NaOH, centrifuged once more and air-dried.

2.3. Characterisation of native and modified starch

Gelatinisation and retrogradation properties of native and modified starches were determined my method described by Babić, Šubarić, Ačkar, Kopjar, and Nedić Tiban (2009), using differential scanning calorimeter DSC 822^{e} (Mettler Toledo) equipped with STAR^{e} software. Starch samples were weighed 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 Rapid Visco-Analyser (Model 803202, Brabender Gmbh & Co KG, Duisburg, Germany). The starch suspensions were equilibrated at 50 °C for 1 min, heated at 7.5 °C/min 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.

Degree of cross-linking (DC) was calculated from RVA data according to method described by Chatakanonda, Varavinit, and Chinachoti (2000) as follows:

$$\mathrm{DC} = \frac{\text{Peak viscosity(native starch)} - \text{Peak viscosity(modified starch)}}{\text{Peak viscosity(native starch)}} \times 100\ (\%) \tag{1}$$

Swelling power (SP) and solubility (SOL) were determined by method described by Babić et al. (2007). Briefly, 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 the following equations:

$$\mathrm{SP} = \frac{\text{weight of gel}}{\text{weight of dry matter in gel}} \left(\frac{\mathrm{g}}{\mathrm{g}}\right) \tag{2}$$

$$\mathrm{SOL} = \frac{\text{weight of dry matter in supernatant}}{\text{weight of dry matter in suspension}}\ (\%) \tag{3}$$

Colour of starch (dry powder) was measured using Chroma Meter CR-300, Konika Minolta, Japan, with granular materials attachment. The instrument was calibrated using white tile and colour was expressed in CIE-Lab parameters as L^* (whiteness/darkness), a^* (redness/greenness) and b^* (yellowness/blueness) and in CIE-LCh parameters as C^* (chroma) and h^* (hue). Five measurements were performed on each sample and mean value and standard deviations were calculated. Colour differences between starch samples were calculated according to the following equation:

$$\Delta E = \sqrt{(L^* - L_n^*)^2 + (a^* - a_n^*)^2 + (b^* - b_n^*)^2} \tag{4}$$

where L^*, a^*, b^* were parameters for modified starch and L_n^*, a_n^*, b_n^* were parameters for native starch.

Paste clarity (in triplicates) was determined by method described by Raina, Singh, Bawa, and Saxena (2006). 1% (d.w.b.) starch suspensions were heated for 30 min in boiling water bath with occasional shaking. After 1 h holding at room temperature % transmittance was read at 650 nm against distilled water as blank.

Freeze–thaw stability was measured by modified method of Lawal (2009). Starch suspension (5% w/w, d.w.b.) was heated in temperature controlled shaking water bath with constant shaking (200 rpm) for 1 h. The paste was weighed (10 g) in pre-weighed PP-centrifuge tubes and subjected to freeze–thaw cycles followed by centrifugation at 4000 rpm for 30 min. Alternate freezing and thawing was performed by freezing for 22 h at −18 °C and thawing for 2 h at 30 °C. Seven freeze–thaw cycles were performed. The weight of water separated after each freeze–thaw cycle was measured and extent of syneresis was calculated by the following equation:

$$\text{Syneresis} = \frac{\text{water separated (g)}}{\text{total weight of sample (g)}} \times 100\ (\%) \tag{5}$$

Starch digestibility was determined by official AOAC Official Method 2002.02 method, using RSTAR Megazyme kit.

Amylose content was determined according to the Megazyme method (Gibson, Solah, & McCleary, 1997). 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 Con A 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 D-glucose, which was analysed using glucose oxidase/peroxidase reagent. Concentration of amylose in starch sample was calculated from the following equation:

$$\text{Amylose} = \frac{\text{Absorbance (Con A)}}{\text{Absorbance (total starch aliquot)}} \times 66.8\ (\%) \tag{6}$$

where 66.8 was dilution factor for Con A 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).

3. Results and discussion

The DSC gelatinisation and retrogradation parameters of native and cross-linked starches are shown in Table 1. Native starch isolated from "Golubica" wheat variety gelatinised at T_o 59.49 °C; T_p 62.68 and T_c 66.66 °C, while native starch isolated from "Srpanjka" had T_o 59.94, T_p 63.72 and T_c 67.82 °C. These temperatures are in accordance with research of Mohan and Malleshi (2006), who determined 59.7, 64.5 and 70.0 °C for aforementioned parameters for common wheat starch and Yoo and Jane (2002) who determined T_o 54.9–55.7 °C, T_p 58.9–62.1 °C and T_c 63.1–67.6 °C for starch from different wheat cultivars.

All modified starches exhibited shift in onset and peak temperature and for "Srpanjka" starch endset temperature. Endset temperature of "Golubica" starch was increased by modification in 0.1 and 0.3% (v/w), but was not influenced by 0.5% (v/w) modification. Carmona-Garcia, Sanchez-Rivera, Mendez-Montealvo, Garza-Montoya, and Bello-Perez (2009) also noted increase in gelatinisation temperatures due to cross-linking with epichlorohydrin. This phenomenon is related to the reduced mobility of amorphous chains in the starch granule as a result of the formation of intermolecular bridges (Singh, Kaur, & McCarthy, 2007).

In "Golubica" variety, 0.3% modification did not have influence on gelatinisation enthalpy (ΔH_g). ΔH_g decreased by 0.1% and 0.5% modification, which is in accordance with research of Carmona-Garcia et al. (2009) and Jyothi et al. (2006). On the basis of DSC studies, Woo and Seib (2002) and Deetae et al. (2008) also reported that the cross-linked starches showed higher gelatinisation temperatures and lower gelatinisation enthalpies than their native counterparts. ΔH_g for "Srpanjka" variety increased following order: S < SE0.3 < SE0.1 < SE0.5. Cross-linking caused increase in ΔH_g values of cross-linked waxy rice starch (Liu, Ramsden, & Corke, 1999), but also decrease of ΔH_g values of cross-linked normal rice starch.

Table 1
DSC gelatinisation and retrogradation properties of starch isolated from wheat varieties "Golubica" (G) and "Srpanjka" (S) and modified with epichlorohydrin (E) in concentrations 0.1%, 0.3% and 0.5% v/w.

	T_o (°C)	T_p (°C)	T_c (°C)	$\Delta H_{g/r}$ (J/g)
Gelatinisation ($n = 3$)				
G	59.49 ± 0.07^{a}	62.68 ± 0.03^{a}	66.66 ± 0.06^{a}	$8.87 \pm 0.07^{c,d}$
GE0.1	59.76 ± 0.07^{c}	63.08 ± 0.07^{e}	66.75 ± 0.08^{a}	8.39 ± 0.03^{a}
GE0.3	$60.23 \pm 0,05^{e}$	$63.58 \pm 0.05^{f,g}$	67.29 ± 0.03^{b}	$8.86 \pm 0.09^{c,d}$
GE0.5	$59.84 \pm 0,04^{e}$	63.17 ± 0.01^{f}	$66.70 \pm 0.10^{a,b}$	8.55 ± 0.05^{b}
S	59.94 ± 0.17^{e}	63.72 ± 0.02^{g}	$67.82 \pm 0.09^{a,b}$	$8.47 \pm 0.01^{a,b}$
SE0.1	60.79 ± 0.02^{b}	64.34 ± 0.06^{b}	73.23 ± 8.72^{a}	8.78 ± 0.09^{c}
SE0.3	60.79 ± 0.05^{d}	64.27 ± 0.06^{d}	68.10 ± 0.10^{a}	8.75 ± 0.06^{d}
SE0.5	$60.74 \pm 0.06^{b,c}$	64.37 ± 0.06^{c}	68.37 ± 0.06^{a}	8.93 ± 0.13^{c}
Retrogradation after 7 days at 4 °C ($n = 3$)				
G	$42.57 \pm 0.29^{c,d}$	52.10 ± 0.26^{a}	60.45 ± 0.05^{a}	3.42 ± 0.01^{c}
GE0.1	$43.67 \pm 0.49^{a,b,c}$	$52.77 \pm 0.57^{b,c}$	$61.10 \pm 0.36^{a,b}$	3.32 ± 0.18^{a}
GE0.3	$43.40 \pm 0.53^{b,c,d}$	$52.88 \pm 0.08^{b,c}$	$60.40 \pm 0.35^{c,d}$	2.91 ± 0.02^{b}
GE0.5	$43.85 \pm 0.19^{a,b}$	$53.27 \pm 0.35^{c,d}$	60.34 ± 0.32^{d}	$2.86 \pm 0.10^{c,d}$
S	43.64 ± 0.42^{a}	$51.67 \pm 0.27^{a,b}$	60.44 ± 0.49^{a}	$3.23 \pm 0.03^{a,b}$
SE0.1	$43.17 \pm 0.31^{c,d}$	$52.57 \pm 0.21^{c,d}$	$60.73 \pm 0.38^{b,c}$	$2.82 \pm 0.04^{c,d}$
SE0.3	$43.30 \pm 0.10^{c,d}$	$52.40 \pm 0.46^{c,d}$	61.53 ± 0.12^{a}	$2.98 \pm 0.02^{c,d}$
SE0.5	42.77 ± 0.21^{d}	52.73 ± 0.38^{d}	62.00 ± 0.26^{a}	3.74 ± 0.01^{d}
Retrogradation after 14 days at 4 °C ($n = 3$)				
G	$42.30 \pm 0.36^{b,c,d}$	$51.40 \pm 0.70^{b,c,d}$	60.74 ± 0.41^{a}	3.81 ± 0.04^{c}
GE0.1	42.43 ± 0.47^{d}	50.87 ± 0.38^{d}	$61.53 \pm 0.32^{a,b}$	3.74 ± 0.06^{e}
GE0.3	$42.05 \pm 0.31^{c,d}$	$51.44 \pm 0.16^{c,d}$	$60.43 \pm 0.21^{b,c}$	3.45 ± 0.09^{f}
GE0.5	$42.19 \pm 0.87^{a,b,c}$	$52.13 \pm 0.28^{a,b,c}$	60.57 ± 0.28^{d}	3.25 ± 0.04^{g}
S	43.14 ± 0.32^{a}	51.55 ± 0.24^{a}	$60.64 \pm 0.06^{a,b}$	3.70 ± 0.04^{d}
SE0.1	$43.53 \pm 0.29^{a,b}$	$52.73 \pm 0.21^{a,b}$	$60.83 \pm 0.06^{c,d}$	$3.97 \pm 0.01^{c,d}$
SE0.3	43.33 ± 0.15^{a}	53.13 ± 0.21^{a}	61.23 ± 0.31^{a}	4.16 ± 0.02^{b}
SE0.5	42.70 ± 0.17^{a}	52.17 ± 0.46^{a}	61.87 ± 0.50^{a}	4.82 ± 0.03^{a}

T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; $\Delta H_{g/r}$, gelatinisation/retrogradation enthalpy. Values are means ± SD of triplicate. Values in the same column with different superscripts (a–g) are significantly different ($P < 0.05$).

It has been reported that ΔH_g of starch increases with increasing level of cross-linking. Cross-linking of starch at lower levels reduces the proportion of starch that can be gelatinised, resulting in lower value of ΔH_g, while higher values of ΔH_g of starches cross-linked at higher levels show complete melting of crystalline regions in spite of cross-linking (Singh et al., 2007).

Cross-linking lowered retrogradation enthalpy after 7 days of storage at 4 °C for both starch varieties modified in 0.1% and 0.3% v/w concentrations, which indicated decreased tendency of modified starch towards retrogradation. After 7 days of storage, 0.5% modification caused decrease of ΔH_r for "Golubica" starch, but increase for "Srpanjka" starch. Tran, Piyachomkwan, and Sriroth (2007) reported that cross-linking decreased tendency of starch towards retrogradation after 8 days of storage.

After 14 days of storage, all modified "Golubica" starches showed lower, while all "Srpanjka" starches showed higher ΔH_r and tendency towards retrogradation. The retrogradation properties of starches are indirectly influenced by the structural arrangement of starch chains, which in turn, influence the extent of granule breakdown during gelatinisation and the interactions that occur between starch chains during gel storage (Jyothi et al., 2006; Singh et al., 2007). While Singh et al. (2007) report that cross-linked starches show lower tendency to retrogradation due to restricted mobility of cross-linked amylopectin branches, Jyothi et al. (2006) report that cross-linking results in ordered structure in the starch pastes, thus resulting in higher degree of retrogradation.

Pasting properties of native and modified starches are shown in Table 2. RVA results showed that cross-linking with epichlorohydrin increased pasting temperature of "Golubica" starch, while it had no significant effect on pasting temperature of "Srpanjka" starch.

Maximum viscosity decreased by cross-linking proportionally to epichlorohydrin concentration in both wheat starch varieties. Maximum viscosity reflects the ability of the granules to swell freely prior to their physical breakdown (Singh et al., 2007). Swelling of starch granules is depressed by enhanced cross-linking which causes viscosity decrease (Kurakake, Akiyama, Hagiwara, & Komaki, 2009). Jyothi et al. (2006) reported drastic decrease of viscosity of cassava starch at epichlorohydrin concentrations 0.75% and 1.0%. Decrease of peak viscosity was also observed for acetylated distarch adipate (Luo, Huang, Fu, Zhang, & Yu, 2009) and starch–carboxylic acid complexes of oxalic, malonic and succinic acids (John & Raja, 1999). Jyothi et al. (2006), however, also reported that influence of cross-linking on maximum viscosity depended on degree of cross-linking – lower degree increased, while higher degree of cross-linking decreased peak viscosity of starch paste. Deetae et al. (2008) reported that reaction time of cross-linking also influenced peak viscosity. As reaction time increased, peak viscosity decreased.

Hot and cold paste viscosities were also decreased by modification with epichlorohydrin for both wheat starch varieties, which is inconsistent with cross-linked starch properties (Luo et al., 2009). However, reaction of epichlorohydrin on starch may result not only in the cross-linking of starch, but also in monoetherification of starch by epichlorohydrin (Hamdi & Ponchel, 1999).

Considerable increase in paste viscosity (measured at 50 °C) caused by cooling of all investigated starches, visible from differences between hot and cold paste viscosities, is due to association of swollen granules, fragments of swollen granules, colloidally dispersed and dissolved starch molecules into larger units (Adebowale, Afolabib, & Olayide, 2002; Singh et al., 2007).

Table 2

Pasting properties of starch isolated from wheat varieties "Golubica" (G) and "Srpanjka" (S) and modified with epichlorohydrin (E) in concentrations 0.1%, 0.3% and 0.5% v/w. Pastes contained 7% starch, w/w (d.w.b.).

	G	GE0.1	GE0.3	GE0.5	S	SE0.1	SE0.3	SE0.5
Pasting temperature (°C)	$64.87 \pm 0.25^{a,b}$	$66.10 \pm 0.30^{a,b}$	$69.75 \pm 0.35^{a,b}$	$69.95 \pm 0.75^{a,b}$	65.60 ± 0.36^{a}	65.75 ± 0.35^{b}	65.60 ± 0.28^{c}	65.70 ± 0.28^{c}
V_{max} (BU)	306.33 ± 5.51^{e}	284.50 ± 4.50^{d}	147.50 ± 5.50^{b}	143.00 ± 4.00^{a}	291.00 ± 3.61^{f}	242.50 ± 4.95^{e}	95.00 ± 1.41^{c}	37.00 ± 0.00^{c}
Viscosity at 92 °C (BU)	256.67 ± 3.79^{h}	224.50 ± 5.50^{e}	100.50 ± 4.50^{b}	87.50 ± 2.50^{a}	272.33 ± 4.16^{g}	200.00 ± 5.66^{f}	60.00 ± 1.41^{d}	26.00 ± 0.00^{c}
Hold 20 min at 92 °C (BU)	240.67 ± 4.16^{d}	283.00 ± 3.00^{e}	150.00 ± 3.00^{b}	142.50 ± 3.50^{a}	214.00 ± 3.00^{e}	241.00 ± 4.24^{f}	95.50 ± 0.71^{c}	37.00 ± 0.00^{c}
Viscosity at 50 °C (BU)	501.67 ± 4.04^{e}	471.50 ± 4.50^{d}	186.50 ± 6.50^{b}	177.50 ± 3.50^{a}	457.67 ± 3.79^{g}	339.00 ± 4.24^{f}	114.50 ± 2.12^{c}	46.00 ± 0.00^{c}
Hold 20 min at 50 °C (BU)	435.00 ± 4.58^{e}	417.00 ± 6.00^{d}	171.50 ± 3.50^{b}	169.00 ± 2.00^{a}	392.33 ± 6.11^{g}	305.00 ± 7.07^{f}	106.00 ± 1.41^{c}	46.50 ± 0.71^{c}
Breakdown	65.67 ± 2.08^{c}	1.50 ± 1.50^{a}	-2.50 ± 2.50^{a}	0.50 ± 0.50^{a}	77.00 ± 2.65^{b}	1.50 ± 0.71^{a}	-0.50 ± 0.71^{a}	0.00 ± 0.00^{a}
Setback	261.00 ± 1.73^{f}	188.50 ± 1.50^{d}	36.50 ± 3.50^{b}	35.00 ± 0.00^{a}	243.67 ± 1.53^{g}	98.00 ± 0.00^{e}	19.00 ± 1.41^{c}	9.00 ± 0.00^{c}
DC (%)		7.13	51.85	53.32		16.67	67.35	87.29

V_{max}, maximum viscosity; breakdown = V_{max}−viscosity at 92 °C after 20 min; setback = viscosity at 92 °C after 20 min−viscosity at 50 °C before holding. Values are means ± SD of triplicate. Values in the same row with different superscripts (a–h) are significantly different ($P < 0.05$).

The breakdown value of starch paste is defined as difference between the peak viscosity and the viscosity after holding at 92 °C for 20 min. It indicates fragility of the granules and stability of starch paste during shearing at high temperatures (Luo et al., 2009). Starches that are capable of swelling to a high degree are also less resistant to breakdown on cooking and hence exhibit significant viscosity decreases after maximum viscosity has been reached (Singh et al., 2007).

Significant lowering of breakdown value by modification of "Golubica" and "Srpanjka" starch with epichlorohydin indicated high stability of modified starch paste during shearing at high temperatures. Similar results were reported by Liu et al. (1999) for cross-linked normal and waxy rice starch. Formation of cross-links between starch molecules strengthen the swollen granules against breakage under conditions of high temperature and shear. With cross-linked starches, the granule may swell as hydrogen bonds are weakened when continuously heated in water. However, after they rupture, the covalent cross-links may provide sufficient granule integrity to keep the swollen granules intact and minimize or prevent loss in viscosity (Jyothi et al., 2006; Lim & Seib, 1993).

Hoover and Vasanthan (1992) explained that setback values reflect the extent of water immobilisation around the charged centres of starch components and those of free and helically complexed lipid molecules (rather than starch paste retrogradation). The association of water molecules with these charged centres would decrease the effective water concentration in the continuous phase, resulting in a rise in viscosity during the cooling cycle. However, setback values can reflect tendency of starch paste towards gel formation (Adebowale et al., 2002) or towards retrogradation (Luo et al., 2009).

Setback values of modified "Golubica" and "Srpanjka" starches were significantly lower than setback values of native counterparts, unlike acetylated distarch adipate (ADA) in research of Luo et al. (2009) and cross-linked starch in research of Tran et al. (2007). Similar results to this research were obtained for octenyl succinic anhydride modified starches (Han & BeMiller, 2007; Song, Xie, Ruan, & Chen, 2006) and hydroxypropylated cross-linked starch (Wattanachant, Muhammad, Mat Hashim, & Rahman, 2003).

Degree of cross-linking was calculated from maximum viscosity values, as shown in Eq. (1). For both starch varieties degree of cross-linking increased with increase of EPI concentration. At all EPI concentrations, degree of cross-linking was higher for "Srpanjka" starch.

Swelling power (SP) and solubility (SOL) (Table 3) increased with increase of temperature for all investigated starches, but increase was more gradual for modified starches, and as reagent concentration was increased. Differences in values of SP and SOL for "Golubica" starch were not statistically different in temperature range 65–85 °C, but at 95 °C modification with epichloroydrin caused significant decrease of both SP and SOL. For "Srpanjka" starch, SP was less influenced at temperatures 65 and 75 °C, but at 85 and 95 °C decrease in SP values was statistically significant. Decrease of swelling power of potato starch cross-linked with epichlorohydrin was observed by Dubois, Picton, Muller, Audibert-Hayet, and Doublier (2001), and decrease of both SP and SOL of cross-linked rice starch was observed by Raina et al. (2006). Similar results were obtained by Luo et al. (2009) for ADA starches and Carmona-Garcia et al. (2009) for epichlorohydrin-modified starches. It has been proposed that bonding forces within the granules of starch affect swelling power. Consequently, highly associated starch granules, with an extensive and strongly bonded micellar structure, should display relatively greater resistance to swelling (Adebowale et al., 2002).

Colour of starches (Table 4) was measured in CIE-Lab and CIE-LCh systems. L^* values in both systems indicate whiteness of starch, which was not statistically different between two native starches. While 0.1% modification did not significantly influence whiteness of "Golubica" starch, 0.3% modification decreased, and

Table 3

Swelling power (SP) and solubility (SOL) of starch isolated from wheat varieties "Golubica" (G) and "Srpanjka" (S) and modified with epichlorohydrin (E) in concentrations 0.1%, 0.3% and 0.5% v/w.

	65 °C		75 °C		85 °C		95 °C	
	SP (g/g)	SOL (%)	SP (g/g)	SOL (%)	SP (g/g)	SOL (%)	SP (g/g)	SOL (%)
G	8.60 ± 0.50^{b}	3.81 ± 0.22^{d}	9.14 ± 0.19^{a}	6.06 ± 0.82^{e}	11.45 ± 0.04^{e}	11.60 ± 0.02^{d}	22.23 ± 0.20^{e}	32.69 ± 1.03^{g}
GE0.1	8.32 ± 0.35^{b}	5.28 ± 0.31^{f}	$8.93 \pm 0.11^{a,b}$	$6.64 \pm 0.31^{c,d}$	10.67 ± 0.04^{d}	$10.82 \pm 0.51^{c,d}$	13.33 ± 0.11^{d}	23.76 ± 0.08^{f}
GE0.3	8.52 ± 0.13^{b}	4.51 ± 0.13^{e}	9.10 ± 0.12^{e}	$5.46 \pm 0.19^{b,c}$	9.74 ± 0.04^{b}	8.32 ± 0.11^{a}	10.62 ± 0.14^{c}	17.31 ± 0.04^{d}
GE0.5	9.28 ± 0.10^{c}	3.77 ± 0.07^{d}	$10.60 \pm 0.01^{a,b}$	$4.70 \pm 0.06^{d,e}$	10.39 ± 0.10^{c}	$10.10 \pm 1.29^{b,c}$	$10.10 \pm 0.12^{b,c}$	15.71 ± 0.17^{c}
S	$8.25 \pm 0.12^{a,b}$	4.53 ± 0.30^{e}	$9.31 \pm 0.02^{b,c}$	6.87 ± 0.42^{e}	11.96 ± 0.17^{f}	13.39 ± 0.04^{e}	23.23 ± 0.65^{f}	35.82 ± 0.24^{h}
SE0.1	$8.23 \pm 0.16^{a,b}$	2.80 ± 0.14^{c}	9.60 ± 0.07^{d}	$5.29 \pm 0.24^{b,c,d}$	10.75 ± 0.11^{d}	$12.11 \pm 0.22^{d,e}$	12.85 ± 0.18^{d}	20.56 ± 0.47^{e}
SE0.3	$8.32 \pm 0.01^{a,b}$	1.92 ± 0.52^{b}	$9.43 \pm 0.05^{c,d}$	4.37 ± 0.06^{b}	9.30 ± 0.17^{a}	$9.13 \pm 0.70^{a,b}$	9.80 ± 0.11^{b}	13.83 ± 0.09^{b}
SE0.5	7.93 ± 0.13^{a}	0.84 ± 0.14^{a}	9.01 ± 0.03^{a}	3.07 ± 0.47^{a}	9.18 ± 0.04^{a}	8.23 ± 0.88^{a}	8.84 ± 0.09^{a}	11.06 ± 0.88^{a}

Values are means ± SD of triplicate. Values in the same column with different superscripts (a–h) are significantly different ($P < 0.05$).

Table 4
Colour (measured in CIELab system) and paste clarity (1% d.w.b.) of starch isolated from wheat varieties "Golubica" (G) and "Srpanjka" (S) and modified with epichlorohydrin (E) in concentrations 0.1%, 0.3% and 0.5% v/w.

	L^*	a^*	b^*	C^*	h^*	ΔE_{native}	% $T_{650\text{ nm}}$
G	75.16 ± 0.05^{b}	$-1.19 \pm 0.01^{c,d}$	$+1.35 \pm 0.01^{a}$	1.80 ± 0.01^{b}	132.00 ± 0.4^{h}		7.8 ± 0.14^{f}
GE0.1	75.20 ± 0.08^{a}	-1.11 ± 0.01^{d}	$+0.47 \pm 0.01^{e}$	1.23 ± 0.03^{c}	156.90 ± 0.4^{d}	0.88	10.2 ± 0.00^{g}
GE0.3	74.77 ± 0.01^{f}	-1.10 ± 0.00^{c}	$+0.64 \pm 0.01^{f}$	1.28 ± 0.01^{d}	150.20 ± 0.4^{c}	0.82	14.9 ± 0.28^{h}
GE0.5	76.61 ± 0.01^{e}	-1.12 ± 0.03^{a}	$+0.74 \pm 0.01^{g}$	1.34 ± 0.02^{e}	146.80 ± 0.3^{b}	1.57	32.2 ± 0.00^{e}
S	76.49 ± 0.02^{b}	-1.24 ± 0.02^{b}	$+1.22 \pm 0.00^{h}$	1.73 ± 0.03^{f}	136.0 ± 0.3^{a}		9.3 ± 0.0^{d}
SE0.1	75.60 ± 0.05^{c}	-1.01 ± 0.01^{f}	$+0.53 \pm 0.01^{c}$	1.14 ± 0.01^{a}	153.2 ± 0.2^{f}	1.15	3.5 ± 0.1^{c}
SE0.3	75.97 ± 0.10^{d}	-1.04 ± 0.01^{e}	$+0.57 \pm 0.01^{d}$	1.20 ± 0.03^{b}	151.8 ± 0.3^{e}	0.86	2.0 ± 0.0^{b}
SE0.5	77.07 ± 0.16^{g}	$-1.10 \pm 0.01^{c,d}$	$+0.51 \pm 0.01^{b}$	1.22 ± 0.02^{b}	155.1 ± 0.3^{g}	0.93	1.3 ± 0.0^{a}

Values are means ± SD of 5 measurements. Values in the same column with different superscripts (a–h) are significantly different ($P < 0.05$).

0.5% modification increased whiteness (L^*). At "Srpanjka" starch, modification in 0.1% and 0.3% concentration of epichlorohydrin decreased whiteness of starch, but 0.5% modification increased it, making positive impact on colour perception.

b^* values are in positive, yellow domain for all investigated starches, which has positive impact on colour perception by human eye.

C^* (chroma) values are used to describe saturation of colour as function of physical brightness of the illumination, or the characteristics of the emitting/reflecting surface. Chroma values range from 0 (completely unsaturated) to 100 (very high chroma or saturation). All investigated starches had low chroma values (1.14–1.80) and, therefore, low saturation of colour, but high proportion of white in colour, which in turn contributed to perception of white by human eye.

Hue (h^*) describes the distinct characteristic of colour that distinguishes red ($h^* = 0°$) from yellow ($h^* = 90°$) from green ($h^* = 180°$) from blue ($h^* = 270°$). Investigated starches had h^* in the range 132–156.9°, which gives combination of green and yellow resulting in grey appearance of colour. Since chroma values are close to one, this grey perception shifts to white and the result is positive perception of starch colour by human eye.

Total colour difference (ΔE) was calculated for modified starches in relation to their native counterparts. According to Jukić, Ugarčić-Hardi, & Koceva Komlenić, 2007, if ΔE is between 0 and 0.5, colour difference is impossible to detect, ΔE 0.5–1.5 is hardly detectable by human eye, ΔE 1.5–3.0 is noticeable by trained people and ΔE 3–6 is detectable by ordinary people. If ΔE is in range 6–12, colour difference is large, but colour group is still the same. $\Delta E > 12$ indicates that samples belong to different colour groups. For "Golubica" starch, ΔE increased following order: GE0.3 (0.82) < GE0.1 (0.88) < GE0.5 (1.57). Of all "Golubica" modified starches, only colour of starch modified with 0.5% epichlorohydin noticeably differed from native starch.

For "Srpanjka" starch ΔE increased following order: SE0.3 (0.86) < SE0.5 (0.93) < SE0.1 (1.15). By human eye, it would be hard to detect colour change caused by modification of "Srpanjka" starch.

Paste clarity of "Golubica" starch, expressed as light transmittance (Table 4), increased following order: native starch (7.8% T) < GE0.1 (10.2% T) < GE0.3 (14.9% T) < GE0.5 (32.2% T). For "Srpanjka" starch, however, paste clarity decreased following same order: native starch (9.3% $T_{650\text{ nm}}$) > SE0.1 (3.5% $T_{650\text{ nm}}$) > SE0.3 (2.0% $T_{650\text{ nm}}$) > SE0.5 (1.3% $T_{650\text{ nm}}$). Paste clarity, expressed by light transmittance, is measure of granule swelling (Craig, Maningat, Seib, & Hoseney, 1989). However, this research as well as van Hung and Morita (2005) established no relationship between swelling power and light transmittance of starch pastes, although van Hung and Morita (2005) reported decrease of light transmittance in cross-linked starches. Lim and Seib (1993) reported increase of light transmittance by cross-linking with STMP and STPP, and Jyothi et al. (2006) reported decrease of light transmittance for epichlorohydrin-modified starch pastes.

Freeze–thaw stability of investigated starches is shown in Fig. 1. It was determined through five cycles of freezing (−18 °C/22 h) and defreezing (30 °C/2 h). Until 4th cycle, native "Srpanjka" starch was more stable than native "Golubica" starch. Between 4th and 5th cycle, it loosed water rapidly, and its syneresis increased significantly, exceeding "Golubica's" syneresis. Out of all investigated concentrations of epichlorohydrin used for modification, only 0.1% v/w somewhat reduced syneresis, while 0.3% and 0.5% v/w significantly increased syneresis during freeze–thaw cycles for both starch varieties.

For "Srpanjka" starch, however, syneresis did not change significantly during freeze–thaw cycles; therefore, high syneresis can be attributed to low swelling power of 0.3% and 0.5% modified starches, rather than low freeze–thaw stability. "Golubica" starch, however, had even more pronounced syneresis in cycles 3–5 than in first cycles and therefore it indeed was destabilised by 0.3% and 0.5% modification.

Jyothi et al. (2006) reported that starches modified with epichlorohydrin were more prone to syneresis during 5 day storage at 4 °C, while Lim and Seib (1993) reported higher stability of phosphorylated starches during three freeze/thaw cycles. Van Hung and Morita (2005), however, reported larger syneresis of cross-linked starches during first two freeze/thaw cycles compared to native counterparts and smaller syneresis during second two freeze/thaw cycles.

Stabilisation of starch paste during freeze–thaw cycles was observed for cross-linked normal rice starch (Liu et al., 1999) and hydroxypropylated cross-linked starches (Van Hung & Morita, 2005; Wattanachant, Muhammad, Mat Hashim, & Rahman, 2002; Wattanachant et al., 2003).

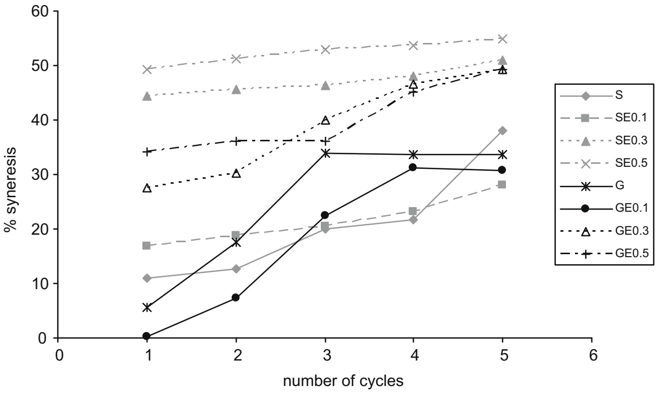

Fig. 1. Freeze–thaw stability of starch isolated from wheat varieties "Golubica" (G) and "Srpanjka"(S) and modified with epichlorohydrin (E) in concentrations 0.1%, 0.3% and 0.5% v/w. Starch pastes (5% d.w.) were kept at −18 °C/22 h and thawed at 30 °C/2 h.

Table 5
Digestibility and amylose content of starch isolated from wheat varieties "Golubica" (G) and "Srpanjka"(S) and modified with epichlorohydrin (E) in concentrations 0.1%, 0.3% and 0.5% v/w.

	NRS (% d.m.)	RS (% d.m.)	TS (% d.m.)	RS/TS (%)	% Amylose
G	$97.15 \pm 1.20^{c,d}$	$0.48 \pm 0.03^{b,c}$	$97.63 \pm 1.22^{b,c}$	$0.49 \pm 0.03^{b,c}$	20.27 ± 0.32^{c}
GE0.1	$95.10 \pm 1.66^{b,c}$	0.31 ± 0.00^{a}	$96.84 \pm 0.07^{a,b,c}$	0.32 ± 0.00^{a}	12.10 ± 1.50^{c}
GE0.3	107.00 ± 1.41^{e}	$0.41 \pm 0.04^{a,b}$	107.44 ± 1.44^{d}	$0.41 \pm 0.02^{a,b}$	12.21 ± 0.85^{b}
GE0.5	98.54 ± 2.26^{d}	0.53 ± 0.09^{c}	99.07 ± 2.21^{c}	$0.54 \pm 0.10^{b,c}$	10.92 ± 0.84^{b}
S	$95.12 \pm 1.18^{b,c}$	0.57 ± 0.11^{c}	$95.70 \pm 1.25^{a,b}$	0.60 ± 0.11^{c}	22.49 ± 2.01^{b}
SE0.1	92.20 ± 0.24^{a}	2.25 ± 0.07^{e}	94.58 ± 0.06^{a}	2.38 ± 0.08^{e}	5.12 ± 0.00^{a}
SE0.3	$93.18 \pm 0.28^{a,b}$	1.24 ± 0.00^{d}	94.42 ± 0.28^{a}	1.31 ± 0.00^{d}	3.36 ± 0.79^{a}
SE0.5	$96.37 \pm 0.43^{c,d}$	1.32 ± 0.07^{d}	$97.69 \pm 0.36^{b,c}$	1.35 ± 0.08^{d}	3.05 ± 0.58^{a}

NRS, non-resistant starch; RS, resistant starch; TS, total starch = NRS + RS. Values are means ± SD of triplicate. Values in the same column with different superscripts (a–e) are significantly different ($P < 0.05$).

Digestibility of investigated starches is shown in Table 5. Both native starches had very low content of resistant starch: 0.49% for "Golubica" and 0.60% for "Srpanjka" (based on total starch amount detected by the method). These values are significantly lower than values Eerlingen, Cillen, and Delcour (1994) determined in wheat starch (1.0% (d.m.) of RS), and Xie, Cui, Li, and Tsao (2008) determined in commercial wheat starch (4.28% RS).

Modification of "Golubica" starch with 0.1% and 0.3% epichlorohydrin increased digestibility, while 0.5% epichlorohydrin did not have significant influence, although it slightly decreased digestibility. Modification of "Srpanjka" starch with epichlorohydrin resulted in reduction of digestibility and twofold increase of resistant starch content by modification with 0.3% and 0.5% epichlorohydrin, and approximately fourfold by 0.1% modification.

Enzymatic degradation of starch by α-amylase is strongly affected by structural characteristics. The process is surface controlled and degradation rate depends on number of blocking groups introduced in hydrogel network (Hamdi & Ponchel, 1999). Some researches showed that cross-linking within level approved for modified food starches had no significant effect on the *in vitro* digestibility of the starch; however, cross-linking interferes with the formation of α-amylase-starch complex (Jyothi et al., 2006).

Amylose content (Table 5) of native "Golubica" starch was 20.27, and of native "Srpanjka" starch 22.49. These results are in accordance with research of Wasserman et al. (2007) and Yoo and Jane (2002). Amylose content was reduced after modification. For "Golubica" starch this reduction was approximately 50%, while for "Srpanjka" it was drastic and approximately 80%. Since amylose was analysed enzymatically, it is possible that this reduction is due to interferences of cross-links with enzymes, moreover due to some properties showed that extent of cross-linking was larger for "Srpanjka" variety, where much less amylose was detected.

4. Conclusions

This research of wheat starches has shown that modification with epichlorohydrin increases temperature of gelatinisation, but gelatinisation and retrogradation enthalpy are affected by the extent of the chemical reaction between starch and epichlorohydrin, due to differences in reactivity between wheat starch varieties. Maximum viscosity decreased as well as breakdown and setback values. Swelling power and solubility were also decreased by modification, while paste clarity and freeze–thaw stability were influenced differently, due to different extent of the chemical reaction between different starch varieties and epichlorohydrin. Colour of starch was not clearly altered by modification. Digestibility of starches can be reduced by proper selection of epichlorohydrin concentration used for modification.

It has been observed that wheat variety has significant influence on starch reactivity towards epichlorohydrin, although it does not significantly influence properties of native starch. Therefore, by selection of proper starch variety and proper epichlorohydrin concentration different desired properties can be achieved.

Wheat starch cross-linked with epichlorohydrin can be used in products where high heat and shear stabilities, as well as improved freeze–thaw stability, are needed.

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