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Effects of autoclaving temperature and storing time on resistant starch formation and its functional and physicochemical properties

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ABSTRACT

In this study effects of autoclaving temperature (140–145 °C) and storing time (24, 48 and 72 h) on resistant starch (RS) formation from high amylose corn starch were investigated and functional and pasting properties of RS preparations were determined. High autoclaving temperature (145 °C) and long storing time (72 h) showed beneficial impacts on RS formation. Significant decreases were observed in all RVA viscosities of RS preparations as the autoclaving temperature increased. There was significant effect of storage time on all RVA parameters of RS preparations within each autoclaving temperature. The water binding values of RS preparations autoclaved at 145 °C were higher than those of the samples autoclaved at 140 °C. RS preparations had approximately 2-fold higher emulsion capacity values than the native starch. Thermal enthalpy (ΔH) values of RS preparations were lower than those of native starch. Autoclaving temperature and storing time had no effects on T_O and T_P.

1. Introduction

Starch is not only one of the primary carbohydrates in human diet (Lehmann & Robin, 2007), but also the most important dietary source of energy for humans (Juansang, Puttanlek, Rungsardthong, Puncha-arnon, & Uttapap, 2012; Lehmann & Robin, 2007; Xie & Liu, 2004). For nutritional purposes, starch is generally classified into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS), depending on the rate and extent of its digestion (Englyst, Kingman, & Cummings, 1992; Song, Janaswamy, & Yao, 2010). Rapidly digestible starch (RDS) is digested in vitro within 20 min, slowly digestible starch (SDS) is digested between 20 and 120 min, and resistant starch (RS) is the starch not hydrolyzed after 120 min of incubation (Englyst et al., 1992).

The resistant starch (RS), escaping hydrolysis by amylolytic enzymes in the small intestine, in an unchanged form is passed to the colon for fermentation where it behaves in a way similar to the dietary fiber. It is subdivided into 4 fractions: RS1, RS2, RS3, and RS4. These are also called as type I, II, III, and IV starches (Haralampu, 2000). RS1 is physically trapped starch, found in partly milled grains, seeds, and legumes. RS2 is condensed and partially crystalline native (ungelatinized) starch granules, that are resistant to the action of enzymes, e.g., in raw potato, banana and high-amylose corn starches. RS1 and RS2 are native starches. They will lose the potential of RS if gelatinized during the processing of food. RS3 consists mainly of retrograded or recrystallized amylose (Garcia-Alonso, Jimenez-Escrig, Martin-Carron, Bravo, & Saura-Calixto, 1999), e.g., in breads, corn flakes, or potatoes. RS4 can be produced by chemical modifications, such as conversion, substitution, or cross-linking. Such modifications prevent digestion of RS4 by blocking access to enzymes and by forming typical linkages. Starch phosphates, hydroxypropyl starches, starch acetates and citrate starches have been tested for enzymatic degradation previously (Wepner, Berghofer, Miesenberger, & Tiefenbacher, 1999).

Resistant starch has physiological effects similar to that of soluble fermentable fiber. RS is not digested in the small intestine, this fraction reaches to the colon and then fermented by beneficial microorganisms in the colon, resulting in the production of short chain fatty acids (mainly acetic, propionic and butyric acids), CO_2, H_2 and in some individuals CH_4, increased fecal bulk and lower colonic pH. Thus, resistant starch decreases colon cancer risk (Dhital, Shrestha, & Gidley, 2010; Garcia-Alonso et al., 1999; Haralampu, 2000; Nugent, 2005; Sajilata, Singhal, & Kulkarni, 2006; Thompson, 2000; Topping & Clifton, 2001; Topping et al., 2003). RS can also behave as a substrate for the growth of the probiotic

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microorganisms (Sajilata et al., 2006; Topping et al., 2003). The consumption of RS reduces glycemic index (GI) value which may have beneficial implications for obesity, type II diabetes, glucose release applications (Fuentes-Zaragoza, Riquelme-Navarrete, Sanchez-Zapata, & Perez-Alvarez, 2010; Ludwig, 2000; Nugent, 2005; Zhou & Lim, 2012). RS also shows promising physiological impact on the prevention of gall stone formation, cardiovascular disease and increasing absorption of minerals (Ca, Mg, Zn, Fe and Cu) (Patindol, Guraya, Champagne, & McClung, 2010; Sajilata et al., 2006; Yue & Wang, 1998).

The RS production methods are usually partial enzyme/acid hydrolysis and hydrothermal treatments, retrogradation, extrusion and chemical modification (Charalampopoulos, Wang, Pandiella, & Webb, 2002). In order to form RS3 from native starch granules (raw starch), the starch has to be gelatinized and retrograded afterwards. During the gelatinization process, the starch granules are gradually and irreversibly destroyed. It is well known that gelatinization temperature has an important influence on RS yields (Escarpa, Gonzales, Morales, & Saura-Calixto, 1997). The autoclaving-mediated formation of RS can be affected by amylose content (Escarpa, Gonzalez, Manas, GarciaDiz, & SauraCalixto, 1996), treatment time, temperature (Onyango, Bley, Jacob, Henle, & Rohm, 2006) and lintnerization (Aparicio-Saguilan et al., 2005). Thus autoclaving has been used to increase RS (Escarpa et al., 1996; Garcia-Alonso et al., 1999; Li, Ward, & Gao, 2011; Mun & Shin, 2006; Ozturk, Koksel, & Ng, 2009; Ozturk, Koksel, & Ng, 2011; Shin, Byun, Park, & Moon, 2004; Simsek & El, 2012; Skrabanja, Liljeberg, Hedley, Kreft, & Bjorck, 1999; Song et al., 2010). Isothermal formation of RS is favored at 100 °C (Eerlingen, Crombez, & Delcour, 1993). High temperature is optimal, but not accessible to 1 atm operations. Thermal cycling to 134 °C is advantageous for the formation of extremely stable RS (Haralampu, 2000).

Starch retrogradation mainly includes short term retrogradation by amylose and long term retrogradation by amylopectin (Haralampu, 2000). During the retrogradation, starch molecules are re-associated and can form tightly packed structures stabilized by hydrogen bonding (Haralampu, 2000). The RS formation is influenced by a number of factors, including the amylose content and chain length of molecules, autoclaving (gelatinization) temperature, storage (retrogradation) time and temperature of starch gels (Eerlingen et al., 1993).

Among the methods used to increase RS yield, acid hydrolysis of amylomaize starch (Chung, Jeong, & Lim, 2003; Lee, Mun, & Shin, 1997a; Man et al., 2012; Ozturk et al., 2011; Sodhi, Chang, Midha, & Kohyama, 2013; Vasanthan & Bhatty, 1998) is the most widely investigated. Mun and Shin (2006) reported that gelatinized and retrograded starch was hydrolyzed easier by acid than native starch. Zhao and Lin (2009) also determined that acid hydrolysis of retrograded high-amylose maize starch at room temperature led to a significant increase in RS yield. Amylopectin might be hydrolyzed by acid to form linear starch molecules, like amylose. Acid treatment of retrograded starch therefore would lead to the increase of RS yield.

There are some investigations on the swelling, solubility and water binding capacity (Koksel, Basman, Kahraman, & Ozturk, 2007; Koksel, Masatcioglu, Kahraman, Ozturk, & Basman, 2008; Koksel, Ozturk, et al., 2008; Mohan, Gopal, Malleshi, & Tharanathan, 2005) and emulsion properties (Koksel, Ozturk, et al., 2008) of RS. A significant increase in water solubility and water binding capacity was observed as a result of heating and autoclaving treatments in RS production (Koksel et al., 2007; Ozturk et al., 2011). Results of investigations show that RS preparations are suitable for food products which require high water binding, good emulsion properties (Kapelko, Zieba, Golachowski, & Gryszkin, 2012; Ozturk et al., 2011).

There are limited studies investigating the functional properties of RS produced by acid modified amylotype starches (Koksel et al., 2007; Koksel, Ozturk, et al., 2008; Ozturk et al., 2011). Furthermore, no information is available on the effect of high autoclaving temperatures (higher than 133 °C) and high storing times (longer than 24 h at 4 °C) on RS formation. There is a need for further research on the effects of different gelatinization and retrogradation parameters on RS formation.

High amylose corn starch is rich in amylose, which makes it very suitable for RS preparation. Therefore, we formed RS from high amylose corn starch by applying autoclaving-cooling (storing) cycles and acid hydrolyzing of gelatinized-retrograded corn starch. The objectives of our study were to investigate (1) the effect of high (140 and 145 °C) autoclaving (gelatinization) temperatures on RS formation; (2) the effects of longer (24, 48 and 72 h at 4 °C) cooling (retrogradation) periods on RS formation; (3) the effects of these on functional properties (solubility, water binding, emulsion capacity and stability) of RS.

2. Materials and methods

2.1. Materials

High amylose corn starch Hylon VII (70%) were obtained from National Starch and Chemical Co. (Bridgewater, NJ). Resistant starch assay kit was purchased from Megazyme International Ireland Ltd. (Wicklow, Ireland).

2.2. Preparation of resistant starch from high amylose corn starch

2.2.1. Autoclaving-cooling cycles

Retrograded amylotype corn starch was prepared according to Zhao and Lin method (Zhao & Lin, 2009) (Fig. 1). High-amylose corn starch (10 g) was mixed with 40 mL of distilled water (starch:water, 1:4), and the mixture was then pressure-cooked in an autoclave at two different temperatures (140 and 145 °C) for 30 min. The autoclaved starch paste was allowed to cool to room temperature and then stored at 4 °C for various periods of time (24, 48 and 72 h). The autoclaving-cooling cycle was repeated three times, then oven dried at 45 °C and grounded, sifted through 212 μm sieve.

In prior studies autoclaving process was made at 133 °C as the highest temperature. In this study, higher autoclaving temperatures than this such as 140 °C and 145 °C were applied.

2.2.2. Acid hydrolysis of retrograded corn starch

Acid hydrolysis was performed according to (Zhao & Lin, 2009) (Fig. 1). Retrograded corn starch prepared by three autoclaving–cooling cycles was dispersed in water in a ratio of 1:4 (w/v). Retrograded corn starch was incubated with 0.1 M HCl in a 40 °C water bath for 24 h. Acid-treated starch slurry was neutralized using 1 M sodium hydroxide. The mixture was stored at 4 °C for 24 h, then oven dried at 45 °C and milled, sifted through 212 μm sieve.

2.3. Determination of resistant starch

Resistant starch content was assessed using the Megazyme Resistant Starch Assay Kit (Megazyme International, Wicklow, Ireland), following the approved AACC method 32-40 (AACC, 2009). Briefly, modified starch (100 mg) and 4 mL of enzyme mixture (pancreatic α-amylase and amyloglucosidase) were added to each test tube, mixture vortexed and then incubated in a shaking water bath for 16 h at 37 °C (200 strokes/min) to hydrolyze digestible starch. At the end of the incubation period suspension was mixed with 4 mL absolute ethanol and vortexed to deactivate the enzymes and RS was recovered as a pellet by centrifugation (5000 × g,

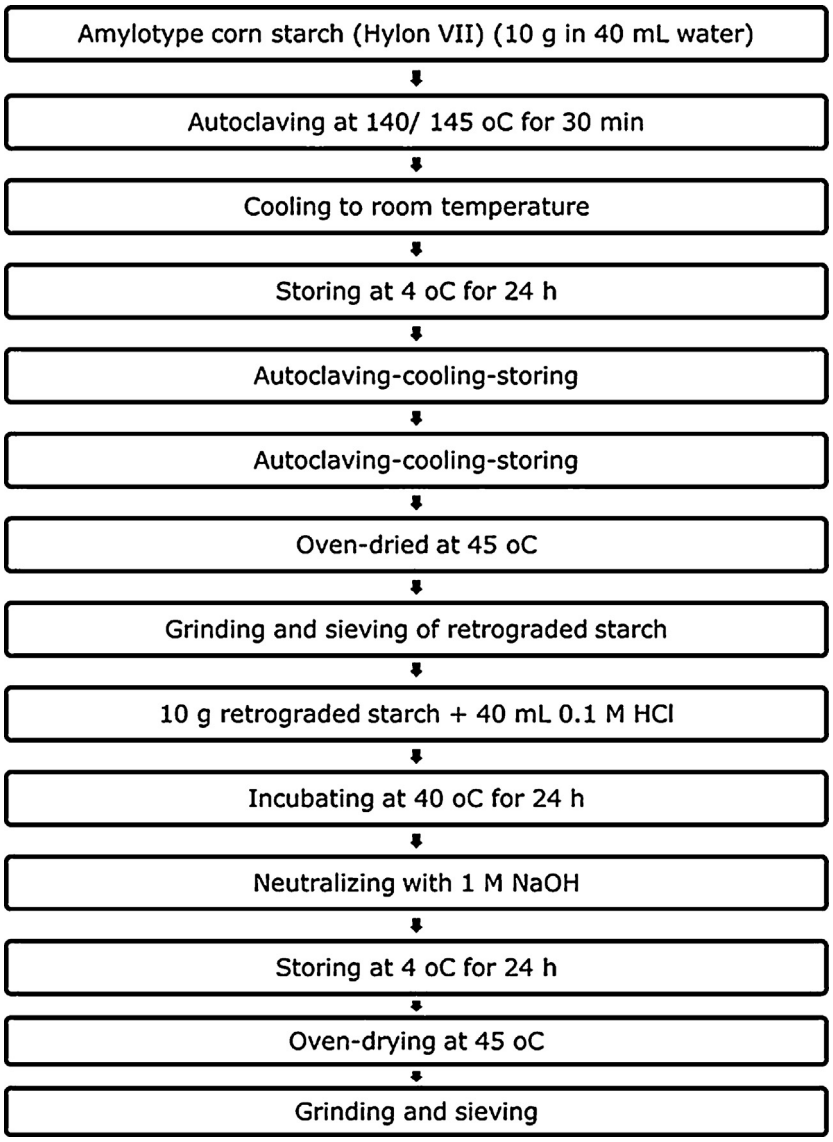

Fig. 1. Preparations of resistant starch.

10 min). Pellet was washed with 50% ethanol twice to remove the digested starch. The sediment was dissolved in 2 mL of 2 M KOH by vigorously stirring for 20 min in an ice bath. This solution was neutralized with 8 mL sodium acetate buffer (1.2 M). Solution was mixed with amyloglucosidase (0.1 mL, 3300 U/mL) and then incubated in a water bath at 50 °C for 30 min, then the samples were centrifuged at 3000 × *g* for 10 min. 3 mL of glucose-oxidase-peroxidase-aminoantipyrine (GOPOD) was added to aliquots (0.1 mL) of the supernatant, and the mixture was incubated at 50 °C for 20 min. Absorbance was measured using a spectrophotometer at 510 nm. Resistant starch and digested starch were calculated as the amount of glucose × 0.9. 162/180 = 0.9 factor to convert from free D-glucose, as determined, to anhydro-D-glucose as occurs in starch (AACC, 2009). Each sample was analyzed in triplicate.

2.4. Functional properties

2.4.1. Water solubility and water absorption index

Water solubility and absorption capacity of the modified starch were determined according to the method previously described by Singh and Singh (2003) with slight modification. The starch (0.5 g, db) was suspended in 5 mL of distilled water and vortexed for 15 s in every 5 min. After 40 min it was centrifuged (Cencom II, Selecta) at 2100 × *g* for 10 min. Supernatant was dried at 100 °C and solubility was calculated as follows:

The water solubility (WS) was calculated as degree of solubility

$$(\%) = \text{grams of solid in supernatant} \times 2/\text{grams of samples} \times 100.$$

Precipitate was weighed and then dried at 100 °C. Water absorption capacity was calculated as follows:

$$\text{Water absorption index (WAI)}\ (\%) = \frac{(\text{weight of wet precipitate} - \text{weight of dried precipitate}) \times 100}{\text{weight of sample}}$$

2.4.2. Emulsifying capacity (EC) and stability (ES)

Emulsifying capacity and stability were determined according to Ahmedna, Prinyawiwatkul, and Rao (1999) and the samples

were prepared according to Abdul-Hamid and Luan (2000) with some minor modifications (Ozturk, Koksel, Kahraman, & Ng, 2009). Starch does not have emulsion properties, but it might affect the emulsion properties of proteins (Herceg, Rezek, Lelas, Kresic, & Franetovic, 2007). Therefore in the present study, effects of starch preparations on the emulsifying properties of albumin solution were investigated. Because of the low emulsifying capacity of modified starch, albumin protein was added to samples. 0.25 and 0.5 g of samples were mixed with 5 mL distilled water or 5 mL of 0.05% albumin protein solution or 2.5 mL distilled water + 2.5 mL of 0.05% albumin protein solution mixture and vortexed for 15 s. Then the solution was mixed with 5 mL of corn oil and homogenized at 23,500 × *g* for 90 s. Then it was centrifuged at 2100 × *g* for 30 min (Cencom II, Selecta). The ratio of the height of the emulsified phase to the height of total liquid was expressed as emulsion capacity (%).

The homogenized samples were incubated at 45 °C for 30 min, after that it was allowed to stand for 10 min at room temperature. Then it was centrifuged at 2100 × *g* for 20 min. The ratio of the height of the emulsified phase to the height of total liquid was expressed as emulsion stability (%). The experiments were conducted in triplicate.

2.5. Pasting properties

The pasting viscosity of modified starches was determined by Rapid Visco-Analyzer (RVA, Newport Scientific, New Brunswick, NJ, USA) according to the AACC method 61-02 (AACC, 1995) with some minor modification. Due to the existing profiles do not correct result, a long profile was applied to be able to observe for differences in pasting properties of high amylose corn starches. The data from the RVA were processed by the Thermocline software, version 1.2 (Newport Scientific Inc., New Brunswick, NJ, USA). Modified starch samples (14%, w/w; 4 g) were weighed into aluminum canisters and mixed with 25 mL water. After putting a stirring paddle into the canister, the canister was placed into the heating chamber. Beginning with rotating the speed of the paddle to 960 rpm for 10 s, modified starch slurries were held at 30 °C for 6 min, heated to 95 °C at a rate of 13 °C/min, held at 95 °C for 20 min, cooled to 40 °C at 11 °C/min, and held at 40 °C for 2 min. A constant rotating speed of the paddle (160 rpm) was used (Ozturk, Koksel, Kahraman, et al., 2009). Pasting temperature, peak viscosity, breakdown, setback, and final viscosity were obtained from viscograms. The results are reported as means of triplicate analyses.

2.6. Thermal properties

The gelatinization characteristics of modified starches were measured using differential scanning calorimetry (DSC, TA 2910, TA Instruments, Newcastle, DE, USA). Modified starch (3 mg, db) was weighed in the stainless-steel large volume pans (PE No. 03190029, Perkin Elmer, MA, USA) and four times greater amount of distilled water was added until the starch was fully wet, and then the container hermetically sealed, allowed to equilibrate for 12 h at 4 °C, as suggested by Miao, Jiang, and Zhang (2009). After equilibration samples were heated at 5 °C/min from 20 to 180 °C to observe the presence of any residual enthalpy gelatinization peak. An empty pan was used as the calibration standard. The gelatinization onset (T_o), peak (T_p), conclusion (T_c) temperatures and the enthalpy change of gelatinization (ΔH) were calculated and recorded using the TA Universal Analysis Software. All measurements were performed in triplicates.

2.7. Statistical analysis

The test data were statistically analyzed using one-way analysis of variance (ANOVA) on SPSS version 17.0 software for Windows (USA). Triplicate determinations were performed for each test for calculation of average and standard derivation. LSD ($p \leq 0.05$) test was applied to determine differences by means of the treatments at the 5% significance level.

3. Result and discussion

3.1. Effect of autoclaving temperature and storing time on the yield of RS

The RS yields of starches, which were autoclaved at 140 and 145 °C and stored at 4 °C for 24, 48 and 72 h are presented in Fig. 2.

The RS yields of the samples autoclaved at 145 °C were significantly ($p \leq 0.05$) higher than those of the samples autoclaved at 140 °C. Lee, Mun, and Shin (1997b) reported that high temperature (121 °C) was more effective than lower temperature (100 °C) in formation of RS in corn starch. Sievert and Pomeranz (1989) reported RS content of 7.0% from corn starch which was treated at 134 °C for 1 h and cooled at 4 °C.

At an autoclaving temperature of 140 °C with 24 h of storage time, the RS content of the starch was 21.73%. Prolonging storing time from 24 to 72 h led to RS content increasing from 21.73 to 26.82%, as a result of longer retrogradation time (Fig. 2).

At an autoclaving temperature of 145 °C with 24 h of storage time, the RS content of the starch was 24.94%. As the storage time was prolonged from 48 to 72 h, the RS content ($p \leq 0.05$) increased significantly. RS yield increased from 24.94% (24 h-storage time) to 30.41% (72 h-storage time), respectively, as shown in Fig. 2. As the storage time was prolonged up to 72 h, the RS content linearly increased. Eerlingen et al. (1993) reported that yields of RS largely depended on storage time and on temperature, and that storage temperature influenced the type of RS crystals (A or B, X-ray diffraction pattern) formed.

3.2. Functional properties

Solubility, water binding capacity, emulsion capacity, and stability of the resistant starch preparations are shown in Table 1.

Resistant starch preparations had higher solubility values than native starch, indicating that these samples had an improving effect on solubility properties. The solubility values of the samples were 4% higher. The solubility values of the autoclaved at 140 °C sample were significantly higher ($p \leq 0.05$) than those of autoclaved at 145 °C sample. The highest solubility value (6.32%) was obtained in the sample prepared with autoclaving at 140 °C and storing for 24 h (Table 1).

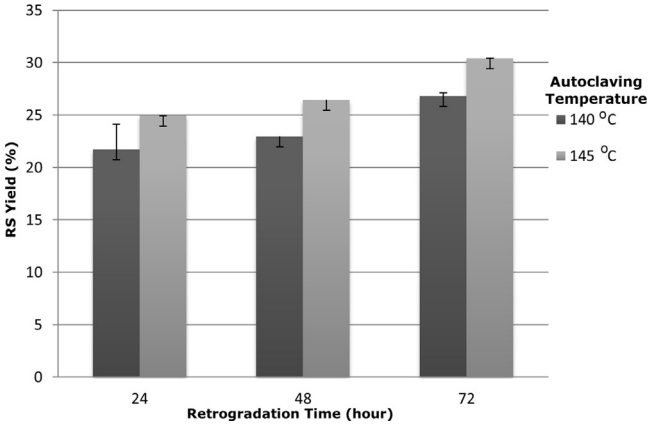

Fig. 2. Resistant starch yields of the samples autoclaved at 140 °C and 145 °C and stored for different periods. Means are based on triplicate analyses.

Table 1
Functional properties of resistant starch preparations.[a]

Samples	Solubility (%)	Water binding (%)	Emulsion capacity (%)	Emulsion stability (%)
Albumin	–	–	22.66 ± 0.39[c]	16.96 ± 1.31[d]
Native starch	2.38 ± 0.23[d]	185.13 ± 0.13[a]	21.54 ± 0.53[c]	45.14 ± 0.13[a]
140 °C/24 h	6.32 ± 0.43[a]	149.56 ± 0.00[e]	51.96 ± 0.51[b]	41.86 ± 1.35[b]
140 °C/48 h	5.76 ± 0.79[ab]	170.17 ± 0.45[c]	53.69 ± 1.50[ab]	41.34 ± 2.73[b]
140 °C/72 h	5.43 ± 0.26[b]	180.46 ± 1.04[b]	56.39 ± 0.48[a]	42.26 ± 1.83[ab]
145 °C/24 h	5.38 ± 0.80[b]	159.83 ± 0.14[d]	55.23 ± 0.58[a]	43.52 ± 1.95[ab]
145 °C/48 h	5.08 ± 0.70[bc]	179.83 ± 0.17[b]	52.75 ± 0.82[b]	45.37 ± 0.96[a]
145 °C/72 h	4.90 ± 0.18[c]	189.76 ± 0.18[a]	51.68 ± 1.84[b]	36.35 ± 2.09[c]

[a] For each sample, means with different letters within each column are significantly different ($p \leq 0.05$).

Significant decreases ($p \leq 0.05$) in solubility values were also observed as the storing time increased from 24 h to 72 h (Table 1). In the samples autoclaved at 145 °C, the highest solubility (5.38%) value was also determined in the sample stored for 24 h.

The highest solubility value (6.32%) was obtained in the sample autoclaved at 140 °C and stored for 24 h (Table 1). Ozturk et al. (2011) determined that in the retrograded acid-hydrolyzed starches, water binding values were in the range of 132–176%, and solubility values were in the range of 0.87–1.29%. As compared with the results of Ozturk et al. (2011), the water binding and solubility values of resistant starch preparations in the present study were higher.

The highest water binding value (189.76%) was obtained in the sample autoclaved at 145 °C and stored for 72 h. The water binding values of RS samples autoclaved at 145 °C were significantly ($p \leq 0.05$) higher than those of the samples autoclaved at 140 °C. The increase in water binding value was mainly due to the gelatinization caused by heating and autoclaving at higher temperature. As the storing time increased, water binding values also significantly ($p \leq 0.05$) increased, except the sample autoclaved at 145 °C and stored for 72 h (Table 1).

Although proteins are frequently used as emulsion forming and stabilizing agents, starch cannot produce emulsion on its own. Starch is in granular form and does not have capacity for remaining at oil–water interface. But it might affect emulsion properties of proteins (Herceg et al., 2007). Therefore, in the present study, effects of various starch preparations on the emulsifying properties of albümin solution were investigated. Emulsion capacity and emulsion stability values of albumin solution ($p \leq 0.05$) were found to be 22.66% and 16.96%, respectively. Emulsion capacity and stability values of albumin supplemented with the resistant starch preparations were significantly ($p \leq 0.05$) higher than those of the albumin solution on its own (Table 1).

High autoclaving temperatures had an improving effect on emulsion capacities of RS preparations compared with native starch. Albumin solution supplemented with resistant starch preparations had approximately 2-fold higher emulsion capacity values than the albumin solution with native starch. There were significant ($p \leq 0.05$) differences between the resistant starch preparations and native starch in terms of the effect on the emulsion capacity. The highest emulsion capacity (56.39%) was obtained in the sample autoclaved at 140 °C and stored for 72 h (Table 1).

Albumin solution supplemented with resistant starch preparations had lower emulsion stability values than the albumin solution with native starch, indicating that these samples had a deteriorative effect on emulsion stability of the albumin. The lowest emulsion stability (36.35%) was determined in the sample autoclaved at 145 °C and stored for 72 h, its water binding value was the highest. The emulsion stability values of the samples autoclaved at 140 °C increased from 41.34% to 42.34% with increasing storing time. In the samples autoclaved at 145 °C, prolonging storing time from 24 h to 48 h also led to an increase in emulsion stability value from 43.52 to 45.37%. However, at the end of the 72 h, the emulsion stability value decrease drastically (36.35%) (Table 1).

There were generally no regular effects of storing time (24, 48, and 72 h) on the emulsion properties of RS preparations within each autoclaving temperature.

3.3. Pasting properties

RVA pasting properties of resistant starch preparations and native starch are given in Table 2 and Fig. 3. Although all RVA parameters, except final viscosity, of RS preparations were significantly ($p \leq 0.05$) higher than those of native ones. Actually, the direct contrary of this was expected. However, the results obtained from this study are in agreement with some previous studies (Koksel et al., 2007; Koksel, Masatcioglu, et al., 2008; Koksel, Ozturk, et al., 2008; Ozturk, Koksel, & Ng, 2009; Ozturk, Koksel, Kahraman, et al., 2009; Ozturk et al., 2011). Ozturk et al. (2011) reported that this is probably caused by excessive degradation of starch granules and increased starch solubility due to the effects of heat and pressure exerted during autoclaving.

The cold peak viscosity values observed in the RVA curves (Fig. 3) were higher than "0" and the viscosity values significantly ($p \leq 0.05$) decreased as the autoclaving temperature and storing time increased. Since the starch is gelatinized prior to RS formation, it is expected to observe a cold peak viscosity value higher than "0" at the initial stage of RVA curve (before heating) (Koksel et al., 2007). Ozturk et al. (2011) reported that the increases in the cold viscosity might be due to the increases in solubility and water binding values.

The RVA viscosity values of the RS preparations significantly ($p \leq 0.05$) decreased as the storing time increased (Table 2).

All RVA viscosity parameters of the RS preparations autoclaved at 145 °C were found to be significantly ($p \leq 0.05$) less than those of the resistant starch autoclaved at 140 °C. The decreases in the viscosity values in the present study might be due to the disrupted starch granules and partial solubilization caused by high autoclaving temperature. Several events occur during the gelatinization of starch: the molecular order and thus birefringence disappears, the starch granule loses crystallinity (melting of crystallites), water is absorbed, granules swell, and when further heated, starch granules are disrupted and partial solubilized (Eerlingen & Delcour, 1995).

3.4. Thermal properties

The thermal properties of native and RS preparations are summarized in Table 3. Although the onset (T_0) and peak (T_p) transition temperatures did not show significant difference between native and RS preparations, ΔH values of all RS preparations significantly

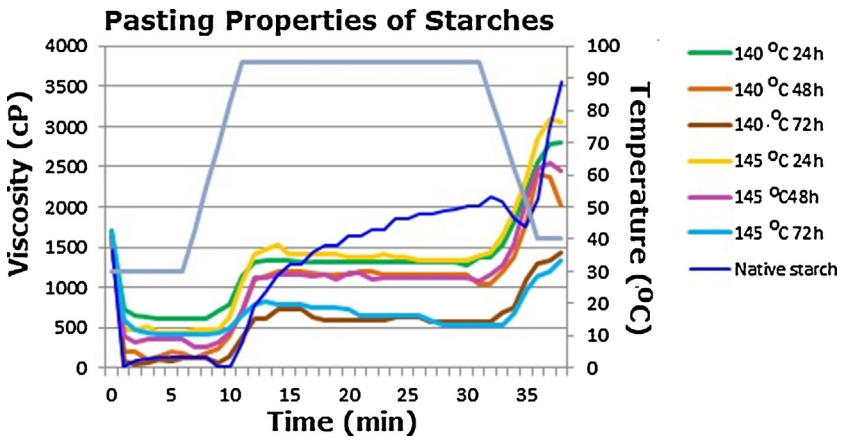

Fig. 3. The pasting curves of resistant starch preparations and native starch (Hylon VII). Means are based on triplicate analyses.

Table 2
RVA pasting properties of resistant starch preparations.[a]

Samples	Cold peak viscosity	Peak viscosity (cP)	Breakdown (cP)	Final viscosity (cP)	Pasting temperature (°C)
Native starch	24 ± 0.13^{g}	324 ± 0.15^{f}	36 ± 0.66^{f}	2952 ± 0.66^{a}	87.5 ± 0.66^{b}
140 °C/24 h	732 ± 0.99^{a}	1536 ± 1.00^{a}	68 ± 1.00^{de}	3096 ± 2.00^{a}	95 ± 0.66^{a}
140 °C/48 h	396 ± 0.33^{c}	1206 ± 0.66^{c}	54 ± 1.00^{ef}	2544 ± 1.32^{c}	95 ± 0.33^{a}
140 °C/72 h	84 ± 0.99^{e}	828 ± 0.66^{d}	168 ± 0.66^{c}	1344 ± 2.00^{e}	95 ± 0.33^{a}
145 °C/24 h	480 ± 0.66^{b}	1332 ± 1.32^{b}	192 ± 1.32^{b}	2784 ± 1.00^{b}	95 ± 0.50^{a}
145 °C/48 h	210 ± 0.66^{d}	1164 ± 0.66^{c}	84 ± 0.33^{d}	2376 ± 2.00^{d}	95 ± 0.66^{a}
145 °C/72 h	58 ± 0.33^{f}	732 ± 2.00^{e}	300 ± 1.65^{a}	1200 ± 1.65^{f}	95 ± 0.33^{a}

[a] For each sample, means with different letters within each column are significantly different ($p \leq 0.05$).

($p \leq 0.05$) decreased as compared to that of the native one (Table 3).

While the highest T_0 value (150.18 °C) was obtained in the sample autoclaved at 140 °C and stored for 48 h. Applying different autoclaving temperature was affected only the onset temperature, the onset temperatures of the resistant starch preparations autoclaved at 145 °C were found to be significantly ($p \leq 0.05$) less than those of the resistant starch preparations autoclaved at 140 °C. Onset (T_0) values of RS preparations within each autoclaving temperature did not show much change for the increase of the storage time.

The highest T_p value (154.72 °C) was obtained in the sample autoclaved at 140 °C and stored for 48 h (Table 3). Compared with those of native starches, T_p values of the RS preparations slightly decreased from 154.69 °C to 151.67 °C, except for the sample autoclaved at 140 °C and stored for 48 h.

Thermal enthalpy (ΔH) values of RS preparations significantly ($p \leq 0.05$) decreased as compared to that of the native starch (Table 3). There were generally irregular effects of storage time on ΔH values of RS preparations within each autoclaving temperature. To observe an increase (not decrease) in the enthalpy values of phase transition of modified starch was expected (Ozturk et al., 2011). However, in this study, ΔH values of RS preparations autoclaved at 140 and 145 °C were lower than that of the native one. These results were very similar to those reported by Ozturk et al. (2011) who emphasized that this might be due to different levels of organization in granular and retrograded starches. It needs to be elucidated with further studies.

Table 3
Thermal properties of resistant starch preparations.[a]

Samples	T_0	T_P	ΔH
Native starch	147.82 ± 1.12^{ab}	154.69 ± 0.35^{a}	14.61 ± 0.53^{a}
140 °C/24 h	147.08 ± 1.73^{ab}	151.67 ± 0.52^{ab}	7.03 ± 1.71^{c}
140 °C/48 h	150.18 ± 1.41^{a}	154.72 ± 0.57^{a}	12.23 ± 1.75^{b}
140 °C/72 h	146.52 ± 1.42^{b}	152.84 ± 1.24^{ab}	7.29 ± 0.50^{c}
145 °C/24 h	144.74 ± 1.15^{b}	151.75 ± 0.87^{b}	10.14 ± 0.40^{bc}
145 °C/48 h	146.82 ± 0.36^{b}	153.85 ± 1.24^{ab}	11.16 ± 0.95^{b}

[a] For each sample, means with different letters within each column are significantly different ($p \leq 0.05$).

4. Conclusions

The results showed that the higher autoclaving temperature (145 °C) and longer storing time (72 h), showed a beneficial impact on RS formation. When high-amylose corn starch was subjected to autoclaving at 145 °C and storing for 72 h, the highest RS yield was obtained. RS preparations had higher solubility values than native starch. As the autoclaving temperatures and storing time increased, water binding values significantly increased. High autoclaving temperatures had an improving effect on emulsion capacities of RS preparations compared with native starch. There were generally no regular effects of storage time on the emulsion properties of RS preparations. Albumin solution supplemented with resistant starch preparations had a deteriorative effect on emulsion stability of the albumin. Significant decreases were observed in all RVA viscosities of RS preparations as the autoclaving temperature increased. There was significant effect of storage time on all RVA parameters of RS preparations within each autoclaving temperature. Cold peak viscosity values decreased as the autoclaving temperature and storing time increased. Although the onset (T_0) and peak (T_p) transition temperatures did not show significant difference between native and RS preparations, ΔH values of all RS preparations significantly

decreased as compared to that of the native one. Compared to the native starch, the RS preparations obtained in the present study seem not to be suitable for the food products, which require relatively higher water binding and cold viscosity and better emulsion stability. Further studies are needed to improve on the functional properties of resistant starch preparations autoclaved at high temperatures.

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