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Formation of resistant starch from amylotype corn starch and determination of the functional properties

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Abstract

High amylose corn starch was hydrolysed with HCl at 40 °C for 0.5-2.0 h. Then the native and acid-modified starch samples were subjected to 2 different heat treatments (HT). In HT1, heat treated/autoclaved samples were dried after storage and in HT2, heat treated/autoclaved samples were dried without storage. Relative quantities of high- and medium-molecular weight fractions decreased and relative quantities of low-molecular weight fractions increased as a result of acid modification. Resistant starch (RS) contents of the acid-modified starches were between 15.7-16.4% and increased up to 29.4% due to HT1 and up to 17.5% due to HT2. The results indicated that RS contents of the high amylose starch can be increased by HT and storing prior to drying. Rapid ViscoAnalayser viscosity values decreased as the acid modification level increased. Water absorption and solubility values of the samples prepared with both heat treatments were higher than those of the native starch and hydrolysates. Emulsifying capacity/stability values of soy protein solution supplemented with the hydrolysates and heat treated samples were higher than those supplemented with the native and heat treated native starch.

Keywords: amylotype starch, emulsifying properties, resistant starch, solubility, water absorption

1. Introduction

Starch in normal corn contains approximately 25% amylose and 75% amylopectin (Shi *et al.*, 1998). Unlike normal starches, the starches from amylose extended mutants of corn contain unusually high amylose, from 55 to 70% (Chung *et al.*, 2003; Patil, 2004). These starches are known as 'amylotype starches'. Amylose content strongly affects the structure and the properties of starch granules (Jiang and Liu, 2002; Patil, 2004). High amylose starches are more resistant to acid/enzyme digestion, has lower swelling power and solubility than normal starches (Jiang and Liu, 2002; Shi *et al.*, 1998). The amylose/amylopectin ratio was reported to affect both gelatinisation and retrogradation of starch (Varavinit *et al.*, 2003). Generally, gelatinisation of normal corn starches occurs at the range of 50-60 °C. However, high amylose starches require higher temperatures than normal starches to adequately gelatinise (Jeong and Lim, 2003; Jiang and Liu, 2002) and require pressurised heating for complete cooking (Chung *et al.*, 2003; Patil, 2004).

Acid modification is widely used in the starch industry to prepare thin boiling starches for use in food, paper, textile and other industries. These modified starches are produced by treating starch with an acid below gelatinisation temperature (Wang *et al.*, 2003). Acid hydrolysis changes the physicochemical properties of starch without destroying its granular structure. Acid modification also increased solubility and gel strength and decreased viscosity of starches (Singh and Ali, 1987; Wang and Wang, 2001; Wang *et al.*, 2003).

Dietary fibers are important food ingredients used in functional foods. There are several studies suggesting the relationship between the consumption of dietary fiber and certain illnesses such as gastrointestinal disease,

hypercholesterolaemia and colorectal cancer (Nasar-Abbas and Jayasena, 2012). Dietary fibers have also great effect in reducing blood glucose level (Al-Dmoor, 2012). Furthermore, fiber addition to the foods decreases the calorie content and the energy intake, which also helps weight control (Nasar-Abbas and Jayasena, 2012).

Resistant starch (RS) has been defined as the fraction of starch, which escapes digestion in the small intestine, and may be fermented in the colon (Haralampu, 2000). Due to the similar health benefits, RS is considered as dietary fiber. In the colon, RS is almost totally fermented to short-chain fatty acids that help to prevent colorectal cancer, lower the risk of heart disease, and improve metabolic and inflammatory bowel diseases such as diabetes and diverticulitis (Sajilata *et al.*, 2006; Sharma *et al.*, 2008). RS is also reported to enhance the ileal absorption of a number of minerals in rats and humans (Lopez *et al.*, 2001; Yunes *et al.*, 1995).

RS is classified into four types according to the mechanism that prevents its enzymatic digestion (Shamai *et al.*, 2003): (1) RS_1 = physically inaccessible starch; (2) RS_2 = native granular starch found in uncooked starch (starches from green bananas, from potatoes or high-amylose corn starch); (3) RS_3 = retrograded starch; and (4) RS_4 = chemically modified starch. The RS_1 and RS_2 are slowly but completely digested with appropriate pre-processing of foods, but RS_3 totally resists digestion (Vasanthan and Bhatty, 1998). RS_3 has high thermal stability which allows it to be stable in normal cooking operations and enables its use as an ingredient in a wide variety of conventional foods (Haralampu, 2000).

The RS_3 contents of foods are generally low; levels up to 3% have been reported in baked foods, pasta and processed cereals and tubers, but can be increased by heat treatments (Vasanthan and Bhatty, 1998). In order to form RS_3, starch has to be gelatinised to disrupt the granular structure and then retrograded for recrystallisation of the starch molecules (Escarpa *et al.*, 1997). During gelatinisation, amylose is leached from the granules into solution as a random coil polymer. Upon cooling, flexible linear amylose polymers begin to reassociate as double helices, stabilised by hydrogen bonds which makes the structure resistant to amylase (Haralampu, 2000; Patil, 2004). Many factors may influence the crystallisation process, and thus the formation of RS, such as amylose content and chain length, autoclaving temperature, storage time and temperature of the starch gels (Eerlingen and Delcour, 1995). High amylose maize starches have very long chains which might be perfectly configured into double helices to form RS (Ozturk *et al.*, 2011). Therefore it is advantageous to use a starch high in amylose to form RS_3.

There are many publications related to the factors affecting RS content in different starches. There are some studies investigating the swelling, solubility and water binding capacity values (Kaur and Singh, 2006; Köksel *et al.*, 2007, 2008a,b; Sandhu and Singh, 2005; Sandhu *et al.*, 2004; Wang *et al.*, 2007) and emulsion properties (Köksel *et al.*, 2008a, b) of regular starches. There are also some studies investigating the functional properties of amylotype starches. Ozturk *et al.* (2009a,b) investigated the effects of debranching, autoclaving-storing and drying processes on pasting and functional properties (solubility, water binding, emulsion capacity and stability) and RS content in amylotype corn starch. The aim of this study was to produce RS preparations from amylotype corn starch with enhanced functional properties (solubility, water binding, emulsion capacity and stability) by using acid modification and different heat treatments. The effects of these treatments on pasting properties of the RS preparations were also investigated.

2. Materials and methods

Materials

High amylose corn starch (Hylon VII) with an amylose content of around 70% was kindly donated by National Starch and Chemical Co. (Bridgewater, NJ, USA).

Acid modification

High amylose corn starch was suspended in diluted HCl (1.64 M) with a starch to HCl solution ratio of 2:3 and incubated at 40 °C for various periods of time (0.5, 1.0, 1.5, 2.0 h). After the incubation period, the pH of the suspension was adjusted to 6 with NaOH (10 g/100 ml). Then the samples were washed three times with distilled water and centrifuged (Heraus Labofuge, Langenselbold, Germany) at 1000 rpm for 5 min (Köksel *et al.*, 2007). The washed samples were dried at 40 °C and ground to pass through 212 µm sieve (Heico, New Delhi, India). Moisture contents of the samples were determined according to AACC approved method 44-15A (AACC, 2000).

Resistant starch formation and determination

For RS formation, native and acid-modified high amylose corn starch samples were subjected to two different heat treatments. In the first one the samples were suspended in water (1:10) and heated in a water bath (100 °C) for 1 h and autoclaved for 30 min at 121 °C (twice). Autoclaved starch samples were stored at 95 °C for 24 h. Then the samples were autoclaved for another 30 min at 121 °C. After the third autoclaving cycle the samples were stored at 95, 50 and 30 °C for 24 h period each and dried at 50 °C (heat treatment 1; HT1). For preparing another set of samples, the samples autoclaved three times (as described above) were dried at 50 °C without storing at different temperatures (heat

treatment 2; HT2). The dried samples were ground and RS contents were determined according to the AOAC method of no. 991.43 (AOAC, 1995). Sequential enzymatic digestion was applied to the samples by using heat stable α-amylase, protease and amyloglucosidase to remove digestible starch and protein. Enzyme digestate was treated with alcohol before filtering, and RS residue was washed with alcohol and acetone, dried and weighed.

Determination of molecular weight distribution

Molecular weight distribution of the native and acid-hydrolysed starch samples were determined by gel filtration technique according to Fredriksson *et al.* (1998) and Han and Lim (2004) with the modifications of Ozturk *et al.* (2009a). As the mobile phase, 0.1 M KOH was used at a flow rate of 0.5 ml/min. Native and acid-modified samples (50 mg) were solubilised in 1 ml 1 M KOH, then KOH concentration was adjusted to 0.1 M with distiled water. Samples at a concentration of 5 mg/ml were applied to Sepharose CL-6B column (1.5×75 cm; Amersham Biosciences, Uppsala, Sweden). The dextrans with the molecular weights of 10, 70, 110, 500 (Amersham Biosciences, Uppsala, Sweden) and 2,000 kDa (blue dextran, Sigma Chemicals Co., St. Louis, MO, USA) were used as molecular weight markers. Fractions (1.5 ml) were collected from the column using a fraction collector (Isco Retriever Model 500; Isco Inc., Lincoln, NE, USA). Total carbohydrate contents of the fractions were determined using the phenol sulphuric acid method (Dubois *et al.*, 1956). Gel filtration chromatograms of the samples were separated into three areas (low-, medium- and high-molecular weight fractions) to compare the molecular weight distributions. The relative quantities of these fractions were calculated by integrating the areas under the curves.

Pasting properties

Pasting properties of the samples were tested by Rapid ViscoAnalyser (RVA 4; Newport Scientific, Warriewood, Australia). In this assay 4 g (14% moisture basis) of native, acid-modified or heat treated amylotype corn starch sample and 25 ml distilled water (adjusted to correct for sample moisture content) were placed in an aluminum sample canister. The RVA pasting curve was obtained by using the 36 min test profile; initial equilibrium at 50 °C for 30 sec, heating to 95 °C over 4 min, holding at 95 °C for 20 min, cooling to 30 °C over 5 min and holding at 30 °C for 6.5 min. The results are reported as means of duplicate analyses.

Functional properties

Solubility and water binding properties of the native, acid-modified and heat treated amylotype corn starch samples were determined using a method based on Singh and Singh (2003) as modified by Ozturk *et al.* (2009a). A 0.5 g sample was added to 5 ml distilled water and vortexed for 15 seconds every 5 min. After 40 min it was centrifuged (Heraus Labofuge, Germany) at 2,100×*g* for 10 min. Supernatant was dried at 100 °C and solubility was calculated as follows:

$$\text{Solubility (\%)} = \frac{\text{weight of dried supernatant}}{\text{weight of sample}} \times 100$$

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

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

Emulsifying capacity and stability were determined according to Ahmedna *et al.* (1999) and the samples were prepared according to Abdul-Hamid and Luan (2000) with some modifications (Köksel *et al.*, 2007). One g of starch sample mixed with 5 ml of 0.05 g/100 ml soy protein solution and vortexed for 15 sec. Then the solution was mixed with 5 ml of corn oil and homogenised at 23,500 rpm for 1 min (Art-Miccra D-8; ART Prozess- & Labortechnik GmbH & Co., Müllheim, Germany). Then it was centrifuged at 2,100×*g* for 20 min. The ratio of the height of the emulsified phase to the height of total liquid was expressed as emulsifying capacity (%). For the determination of emulsifying stability, homogenised sample was incubated at 45 °C for 30 min, rested at room temperature for 10 min and then centrifuged at 2,100×*g* for 20 min. The ratio of the height of the emulsified phase to the height of total liquid was expressed as emulsifying stability (%).

Statistical analysis

The data on functional properties (solubility, water binding and emulsifying capacity and stability properties) and RS content analysed using one-way analysis of variance (ANOVA). When significant ($P<0.05$) differences were found, Duncans' test was used to determine the differences among means.

3. Results and discussion

Molecular weight distribution

Relative quantities of the different molecular weight fractions of native and acid modified amylotype corn starch samples are presented in Table 1. Gel filtration chromatograms of the samples were separated into three areas: high (5,300-2,200 kDa), medium (2,200-770 kDa) and low molecular weight (770-4 kDa) fractions to compare the molecular weight distributions. Gel filtration results indicated that the relative quantities of the high- and medium-molecular weight fractions decreased as the hydrolysis time increased. The decrease in the relative

Table 1. Relative quantities of the different molecular weight fractions of native and acid modified amylotype corn starch samples.

Hydrolysis time (h)	High-molecular weight fraction (%) (5,300-2,200 kDa)	Medium-molecular weight fraction (%) (2,200-770 kDa)	Low-molecular weight fraction (%) (770-4 kDa)
0 (native)	8.6	42.8	48.6
0.5	5.4	42.6	52.0
1.0	3.7	40.7	55.6
1.5	3.2	37.3	59.5
2.0	3.2	33.5	63.2

quantities of high- and medium-molecular weight fractions resulted in an increase in the relative quantities of low-molecular weight fractions. Similar results were obtained in the related literature (Chang *et al.*, 2004; Köksel *et al.*, 2008b; Ozturk *et al.*, 2011; Singh and Ali, 2000).

Resistant starch content

RS contents of the native, acid-modified and heat treated samples are shown in Table 2. RS content of native Hylon VII was 16.1%. RS contents of acid-modified starches were between 15.7-16.4% (dry basis) and the differences between the RS contents of native and acid-hydrolysed samples were not statistically significant (Table 2).

In this study native and acid-modified starch samples were subjected to two different heat treatments (HT). In the first one, the samples were autoclaved three times (as described in the 'Materials and methods' section) were stored at 95, 50 and 30 °C for 24 h period each and dried at 50 °C (HT1). For preparing another set of samples, the samples autoclaved three times were dried at 50 °C without storing (HT2). Heat treatment 1 caused substantial increases in the RS contents as compared to those of the native and acid-modified starch samples. There were no statistically significant differences in RS contents of the native starch sample and the samples hydrolysed for 0.5 and 1.0 h and then subjected to HT1 (Table 2). The highest RS content was achieved at the sample hydrolysed for 2.0 h prior to HT1 (29.4%). RS contents of the samples prepared by heat treatment 2 were between 17.1-17.5% (dry basis) and the differences between the RS contents of samples hydrolysed at different levels were not statistically significant (Table 2). The higher RS contents of the samples prepared by HT1 are probably due to suitable conditions for retrogradation of starch during drying at 50 °C. Throughout the drying period in an oven, starch molecules can reassociate and form tightly packed structures stabilised by hydrogen bonding. These compact molecular structures limit the accessibility of digestive enzymes (Haralampu, 2000).

Table 2. Resistant starch (%, dry basis) contents of the native, acid-modified and heat treated samples.

Hydrolysis time (h)	Hydrolysis	Hydrolysis + HT1	Hydrolysis + HT2
0 (native)	16.1[a]	25.4[b]	17.2[a]
0.5	15.7[a]	25.8[b]	17.1[a]
1.0	15.9[a]	25.7[b]	17.1[a]
1.5	16.4[a]	29.3[a]	17.5[a]
2.0	16.1[a]	29.4[a]	17.5[a]

HT1 = heat treated/autoclaved samples were dried after storage; HT2 = heat treated/autoclaved samples were dried without storage. Means with different superscript letters within each column are significantly different (P<0.05).

During the earlier steps of RS determination by the AOAC method of no. 991.43 (AOAC, 1995), the samples are digested by heat stable α-amylase at 100 °C. While RS2 can be slowly but completely digested after thermal processing, RS3 resists digestion (Escarpa *et al.*, 1997). Hylon VII sample without heat treatment is in granular form and expected to include only RS2 (Brown, 1996; Nugent, 2005). Therefore RS contents determined in the Hylon VII samples (native and acid hydrolysed starches) are probably RS2 which is not completely digested during incubation with heat stable α-amylase in RS determination.

Pasting properties

RVA pasting properties of the native, acid-modified and heat treated samples are shown in Table 3. The gelatinisation temperature of the Hylon VII starch is very high and reported to be in the range of 154-171 °C (Jeong and Lim, 2003; Jiang and Liu, 2002; Ozturk *et al.*, 2011; Xie and Liu, 2004). Therefore, the samples used in this study did not seem to be fully gelatinised during RVA analysis and very low viscosity values were observed. This result agrees with those reported in the literature (Jeong and Lim, 2003; Jiang and Liu, 2002; Ozturk *et al.*, 2011; Song and Jane, 2000).

Table 3. Rapid ViscoAnalyser pasting properties of the native, acid-modified and heat treated samples.

Treatment	Hydrolysis time (h)	Pasting properties		
		Peak viscosity (cP)	Breakdown (cP)	Final viscosity (cP)
Hydrolysis	0 (native)	388[a]	137[a]	876[a]
	0.5	147[b]	26[b]	351[b]
	1.0	117[c]	25[b]	270[c]
	1.5	80[d]	16[b]	203[d]
	2.0	66[d]	16[b]	156[e]
Hydrolysis + HT1	0 (native)	475[a]	68[a]	958[a]
	0.5	295[b]	37[b]	441[b]
	1.0	201[c]	39[b]	303[c]
	1.5	151[d]	38[b]	194[d]
	2.0	115[e]	15[c]	180[e]
Hydrolysis + HT2	0 (native)	533[a]	158[a]	955[a]
	0.5	460[b]	90[b]	851[b]
	1.0	314[c]	84[b]	579[c]
	1.5	241[d]	74[c]	309[d]
	2.0	156[e]	42[d]	270[d]

HT1 = heat treated/autoclaved samples were dried after storage; HT2 = heat treated/autoclaved samples were dried without storage.
For each treatment, means with different superscript letters within each column are significantly different (P<0.05).

Similar to the results obtained by Chung *et al.* (2003) and Ozturk *et al.* (2011) the RVA viscosity values of the samples decreased as the level of acid modification increased. The major effect of acid on starch molecules is the reduction of the molecular weight. The gel filtration results indicated that the molecular weight of starch molecules decreased as the hydrolysis time increased (Table 1). The decreases in the viscosity values due to acid hydrolysis might have been caused because of reduced molecular weight (Köksel *et al.*, 2008b; Wang and Wang, 2001).

RVA viscosity values of the samples prepared by the HT1 and HT2 were found to be higher than those of the hydrolysates (Table 3). This is probably caused by excessive degradation of starch granules due to the effects of heat and pressure exerted during autoclaving. These degraded starch particles can easily intake water during RVA analysis as compared to Hylon VII and the hydrolysates which result in higher viscosity values. RVA viscosity values of the heat treated samples dried without storage (HT2) were higher than those of the heat treated samples dried after the storage (HT1). This is probably an effect of retrogradation process which occurs in the storage period. Retrogradation induces H-bond formation and higher extent of H-bond limits the water intake to the structure during RVA analysis, therefore RVA viscosity values of the stored samples (HT1) are higher than those of the unstored ones. All of the RVA viscosity values of the samples prepared with heat treatment decreased considerably as the level of acid modification increased.

Functional properties

Water absorption and solubility values of the samples are shown in Table 4 and 5, respectively. Water absorption and solubility values of the native starch were found to be lower than those of the hydrolysates (P<0.05) and these properties did not change significantly with the hydrolysis time. The heat treated samples had greater water absorption and solubility values than those of the native starch and hydrolysates. The water absorption values of the heat treated native starch samples and the heat treated hydrolysates within each heat treatment group were not significantly different.

There are some studies investigating the solubility and water binding capacity values of different starches. Kaur and Singh (2006) found that solubility values of starches from different chickpea cultivars varied from 13.2 to 14.9%. Solubility and water binding capacity values of the *Fritillaria* starches were in the range of 10.4-21.3% and 131.4-134.7%, respectively (Wang *et al.*, 2007). Sandhu *et al.* (2004) reported that solubility and water binding capacity of starches from different corn types ranged from 12.5 to 20.3% and 96%

Table 4. Water absorption values (%, dry basis) of the native, acid-modified and heat treated samples.

Hydrolysis time (h)	Hydrolysis	Hydrolysis + HT1	Hydrolysis + HT2
0 (native)	119.8[b]	185.9[a]	187.2[a]
0.5	132.4[a]	183.8[a]	189.0[a]
1.0	131.6[a]	186.8[a]	185.6[a]
1.5	130.7[a]	183.5[a]	186.0[a]
2.0	131.3[a]	181.8[a]	186.7[a]

HT1 = heat treated/autoclaved samples were dried after storage; HT2 = heat treated/autoclaved samples were dried without storage. Means with different superscript letters within each column are significantly different (P<0.05).

Table 5. Solubility values (%, dry basis) of the native, acid-modified and heat treated samples.

Hydrolysis time (h)	Hydrolysis	Hydrolysis + HT1	Hydrolysis + HT2
0 (native)	0.14[b]	0.59[c]	0.53[b]
0.5	0.45[a]	0.79[b]	0.73[a]
1.0	0.51[a]	0.80[b]	0.74[a]
1.5	0.52[a]	0.96[a]	0.75[a]
2.0	0.54[a]	0.95[a]	0.78[a]

HT1 = heat treated/autoclaved samples were dried after storage; HT2 = heat treated/autoclaved samples were dried without storage. Means with different superscript letters within each column are significantly different (P<0.05).

Table 6. Emulsion capacity (%) of the native, acid-modified and heat treated samples.

Hydrolysis time (h)	Hydrolysis	Hydrolysis + HT1	Hydrolysis + HT2
Soy protein (0.05%)	16.7[b]	16.7[b]	16.7[b]
0 (native)	4.6[c]	6.8[c]	5.3[c]
0.5	53.2[a]	54.9[a]	54.9[a]
1.0	55.1[a]	55.6[a]	53.9[a]
1.5	55.6[a]	53.9[a]	53.0[a]
2.0	55.1[a]	54.5[a]	52.8[a]

HT1 = heat treated/autoclaved samples were dried after storage; HT2 = heat treated/autoclaved samples were dried without storage. Means with different superscript letters within each column are significantly different (P<0.05).

Table 7. Emulsion stability (%) of the native, acid-modified and heat treated samples.

Hydrolysis time (h)	Hydrolysis	Hydrolysis + HT1	Hydrolysis + HT2
Soy protein (0.05%)	12.5[b]	12.5[b]	12.5[b]
0 (native)	5.4[c]	4.3[c]	6.7[c]
0.5	55.1[a]	58.5[a]	60.2[a]
1.0	55.8[a]	57.3[a]	58.6[a]
1.5	53.1[a]	56.3[a]	59.0[a]
2.0	54.0[a]	56.3[a]	59.3[a]

HT1 = heat treated/autoclaved samples were dried after storage; HT2 = heat treated/autoclaved samples were dried without storage. Means with different superscript letters within each column are significantly different (P<0.05).

to 107%, respectively. The water binding capacity results of native and acid hydrolysed samples used in the present study were found to be comparable with those of Sandhu *et al.* (2004) and Wang *et al.* (2007). However, water binding capacity values of the heat treated samples were higher.

Proteins are commonly used as emulsion forming and stabilizing agents. On the other hand, starch cannot produce emulsion by itself, but might affect emulsion properties. Therefore, in the present study, effects of the native starch and all starch preparations on the emulsifying properties of soy protein solutions were investigated. Emulsifying capacity and stability properties of the samples are shown in Table 6 and 7, respectively. Emulsifying capacity and emulsifying stability values of soy protein solution (0.05%) were found to be 16.7% and 12.5%, respectively. Soy protein solution supplemented with the native starch and the heat treated native starch samples had emulsifying capacity values (4.6-6.8%) less than the soy protein solution on its own. The results indicated that the native and the heat treated native starch samples affected the emulsion properties of the soy protein inversely. On the other hand, emulsifying capacity values of soy protein solution supplemented with the hydrolysates and the heat treated hydrolysates were found to be significantly higher than those supplemented with the native starch and the heat treated native starch samples within each column (P<0.05). Emulsifying stability values of soy protein solution supplemented with the native and the heat treated native starch samples were in the range of 4.3-6.7%. Emulsifying stability values of soy protein solution supplemented with the hydrolysates (53.1-55.8%) and the heat treated hydrolysates were higher (56.3-60.2%) than those of the native and the heat treated native starch samples. Although, the native and heat treated native starch

samples had a deteriorating effect on emulsion properties of the soy protein solution, hydrolysates and the heat treated hydrolysates improved emulsion capacity and stability values of the soy protein solution significantly ($P<0.05$).

In a previous study of our group, native (containing 25% amylose) and acid modified corn starch samples affected the emulsion properties of the soy protein inversely. RS preparations produced from normal corn starch did not seem to have an improving effect on emulsion properties of the soy protein solution (Köksel *et al.*, 2007). On the other hand, the results of the present study indicated that both acid hydrolysed and/or heat treated amylotype corn starch had improving effect on the emulsion properties of soy protein solution. Similar findings were also reported by using whey and different carbohydrates (Herceg *et al.*, 2007), soy protein and starch (Köksel *et al.*, 2008a,b), albumin and starch (Ozturk *et al.*, 2011). In another study, RS preparations, produced from normal corn starch with lyophilisation prior to acid modification, had a substantial improving effect on emulsion properties of soy protein solution (Köksel *et al.*, 2008a). Acid-modified starches had higher water binding values, therefore they tended to remain suspended in the emulsion at the oil-water interface and precipitated to a lower extent upon centrifugation applied after emulsion formation, and hence, did not cause deterioration in the emulsion properties of albumin solution (Chang *et al.*, 2004; Ozturk *et al.*, 2011).

4. Conclusions

Food industry seems to have a growing interest to enhance the RS content of foods due to its beneficial physiological effects, similar to those of dietary fibre. There are various studies investigating the factors which influence the formation and amount of RS, such as amylose content and chain length, autoclaving temperature, storage time and temperature of the starch gels. In this study it was decided to produce greater levels of RS from high amylose corn starch and determine the functional properties of these starch samples. The results showed that the heat treatment increased RS contents to around 30% and functional properties (especially emulsion properties) were improved. The samples prepared by HT1 had higher RS contents, probably due to ongoing retrogradation of starch during storage period. Throughout the retrogradation period, starch molecules may re-associate and form tightly packed structures stabilised by hydrogen bonding, with loosely rearranged regions among these tightly packed structures. This study has shown that RS containing samples could be produced from acid-modified high amylose corn starch. The samples with high RS content obtained in the present study seem to be suitable for the food products which require good emulsifying properties. Further studies are needed to produce starch preparations with high RS content and improved functional properties.

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