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EFFECT OF ACID MODIFICATION AND HEAT TREATMENTS ON RESISTANT STARCH FORMATION AND FUNCTIONAL PROPERTIES OF CORN STARCH

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In this study effects of acid hydrolysis, autoclaving and storage (95°C for 2, 3, and 4 days) on resistant starch (RS) formation in corn starch were investigated and functional properties of RS preparations were determined. RVA peak and final viscosity values of the RS preparations decreased gradually for each storage period with increasing hydrolyzation level. RS contents increased to 13.6–16.7% as a result of storage. RS contents of the samples dried without storage were lower than those of the stored samples up to 3.5 h hydrolysis. Solubility and water binding values of RS preparations were higher than those of the native and hydrolysed samples. In contrast to RS preparations, the native and hydrolysed samples affected the emulsion properties of soy protein inversely.

Keywords: ***Resistant starch, Water binding, Solubility, Emulsion properties, RVA.***

INTRODUCTION

Resistant starch (RS) is the starch fraction that is not hydrolyzed in the small intestine but may be fermented in the colon. Due to its similar physiological properties, it is generally considered as a constituent of dietary fiber. RS contents in food range approximately between 0–4%. A higher amount of RS in the daily diet is recommended due to its preventative and therapeutical health effects.[1] RS is a fermentation substrate for colonic bacteria and hence has potential for prebiotic applications. Fermentation of RS produces short-chain fatty acids such as acetate, propionate and butyrate. These fatty acids lower the overall pH of the colon, induce chemoprotective enzyme activity and hinder growth of harmful colonic bacteria and thus play a role in protecting against colorectal cancer. Other benefits of RS include lowering of plasma cholesterol and blood lipids as well as improving glucose tolerance.[2,3]

There are four types of resistant starches. RS1: physically inaccessible starch locked within cell walls, RS2: native granular starch, RS3: retrograded or crystalline starch and RS4: chemically modified starches.[4–7] RS3 is the most common in the human diet because it is formed mainly as a result of food processing.[8] Processing of raw materials

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in most cases destroys RS1 and RS2, but it can produce RS3. Most researches suggest that RS formed during processing is associated with amylose retrogradation.[9,10] The RS1 and RS2 are slowly but completely digested with appropriate pre-processing of foods, but RS3 totally resists digestion. The RS3 contents of foods are generally low; levels up to 3% have been reported in baked foods, pasta and processed cereals. RS3 content of starches can be increased by heating and cooling cycles.[11] Flexible linear amylose molecules align themselves after gelatinization into tight linear configurations that form helices, making many of the α-1,4 glucosidic linkages inaccessible to amylase.[7] Many factors may influence the crystallization process, and thus the formation of RS, such as amylose content and chain length, autoclaving temperature, storage time and temperature of the starch gels.[12]

Food applications of resistant starch are of interest to product developers and nutritionists mainly due to fiber-fortification and the potential physiological benefits as well as unique technological properties. RS gives better cereal products, especially in terms of texture, mouthfeel, flavor, color and mineral bioavailability which are not attainable with traditional insoluble fibers.[11,13,14] Besides their nutritional value starches have been used for their functional properties. There are some studies investigating the swelling, solubility and water binding capacity values[15,16,17] and emulsion properties[18,19] of various starches. However, to the best of our knowledge, there are no studies investigating the effects of RS preparations on emulsion properties. The objective of this study was to investigate the effects of acid hydrolysis, heat treatment and subsequent storage on RS formation in corn starch. Functional properties of the RS preparations were also investigated.

MATERIALS AND METHODS

Materials

Normal corn starch with an amylose content of around 25% was obtained from Cargill Inc., Istanbul, Turkey.

Acid Modification

Corn starch (80 g) was suspended in 120 mL 1.64 M HCl and incubated at 40°C for various periods of time (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 h). After the incubation period, the pH of the suspension was adjusted to 6 with 10% NaOH. Then the samples were washed three times with distilled water and centrifuged (Heraus Labofuge, Germany) at 1000 rpm for 5 min.[20] The washed samples were dried at 40°C and ground to pass through 212 μm sieve. Moisture contents of the samples were determined according to the standard AACC method.[21]

Resistant Starch Formation

For resistant starch formation, a set of four samples for native and acid hydrolyzed starch samples were suspended in water (1:10) and gelatinized at 85°C for 15 min. Then they were autoclaved at 121°C for 30 min and three samples from each set were stored at 95°C for 2, 3, and 4 days. They were dried at 50°C, ground and sifted through 212 μm sieve. The fourth sample of each set was dried after autoclaving without storage and used as the control of the set. RS contents of the samples were determined by the enzymatic-gravimetric procedure.[22]

Pasting Properties

Pasting properties of the samples were tested using a Rapid ViscoAnalyzer (RVA 4, Newport Scientific, Australia). A 4 g sample and 25 g distilled water were placed in an aluminum sample canister. The RVA pasting curve was obtained by using a 30 min test profile; initial equilibrium at 30°C for 10 min, heating to 95°C over 6 min, holding at 95°C for 5 min, cooling to 50°C over 5 min, and holding at 50°C for 4 min. The peak viscosity, breakdown viscosity and final viscosity values were evaluated with the data analysis software (Thermocline for Windows, Newport Scientific, Australia).

Functional Properties

Solubility values of the samples were determined using a method based on Singh and Singh.[23] A 0.5 g sample was added to 5 mL distilled water and vortexed for 15 sec every 5 min. After 40 min it was centrifuged (Heraus Labofuge, Germany) at 600 xg for 10 min. Supernatant was dried at 100°C and solubility was calculated as follows:

$$\text{Solubility (\%)} = \frac{\text{weight of dried supernatant}}{\text{weight of sample}} \times 100. \quad (1)$$

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

$$\text{Water binding capacity (\%)} = \frac{\text{weight of wet precipitate} - \text{weight of dried precipitate}}{\text{weight of sample}} \times 100. \quad (2)$$

For the determination of fat binding capacity, 1 g sample was mixed with 10 mL corn oil in a centrifuge tube and vortexed for 1 min. After holding a period of 30 min, the tube was centrifuged (Heraus Labofuge, Germany) at 2100 xg for 10 min. Fat binding capacity was expressed as the amount (ml) of oil bound by 100 g sample.[24]

Emulsion capacity and stability values were determined according to Ahmedna et al. [25] and samples were prepared according to Abdul-Hamid and Luan.[26] Five mL of 7% dispersion of the starch sample (prepared with 0.05% soy protein solution) was mixed with 5 mL of corn oil and homogenized at 23 500 rpm for 1 min (Art-Miccra D-8, Germany). Then it was centrifuged (Heraus Labofuge, Germany) 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 capacity (%). For the determination of emulsion stability, homogenized sample was incubated at 45°C for 30 min and then centrifuged (Heraus Labofuge, Germany) at 2100 xg for 20 min. The ratio of the height of the emulsified phase to the height of total liquid was expressed as emulsion stability (%).

Statistical Analysis

Data were analyzed for variance using the MSTAT-C statistical program. When significant differences were found, the LSD (Least Significant Difference) test was used to determine the differences among means.[27]

RESULTS AND DISCUSSION

Pasting Properties

RVA pasting properties of native and acid modified starch samples are presented in Table 1 and the pasting curves of selected acid modified starch samples (1, 2, 3, and 4 h hydrolysed) are shown in Figure 1. All of the viscosity values of acid modified starch samples were found to be less than those of the native starch. As the level of acid modification increased, all of the RVA viscosity values of the hydrolysates significantly ($p < 0.05$) decreased. As indicated in the related literature, acid hydrolysis causes reduction in the molecular weight of starch.[28] The decreases in the viscosity values observed in the present study might be due to the reduced molecular weight caused by acid hydrolysis.

RVA pasting properties of RS preparations are presented in Table 2 and the pasting curves of selected samples (native and 1, 2, and 4 h hydrolysed, stored for 0 and 3 days) are shown in Figure 2. Peak and final viscosity values of the RS preparations decreased gradually for each storage period as the level of acid modification increased (Table 2). In general, RVA viscosity values of the samples dried without storage were significantly ($p < 0.05$) higher than those of the samples stored for 2, 3, and 4 days. The breakdown and final

Table 1 Pasting properties of native and acid modified corn starch samples.

Hydrolysis time (hr)	Peak viscosity (cP)	Breakdown (cP)	Final viscosity (cP)
Native starch	5886 a	3418 a	5929 a
0.5	4372 b	3253 b	2930 b
1.0	3240 c	2557 c	1664 c
1.5	2426 d	2055 d	974 d
2.0	1827 e	1603 e	688 e
2.5	1221 f	1092 f	495 e
3.0	417 g	365 g	234 f
3.5	369 g	332 g	197 f
4.0	123 h	109 h	112 f

Means with the same letter within each column are not significantly different ($p < 0.05$).

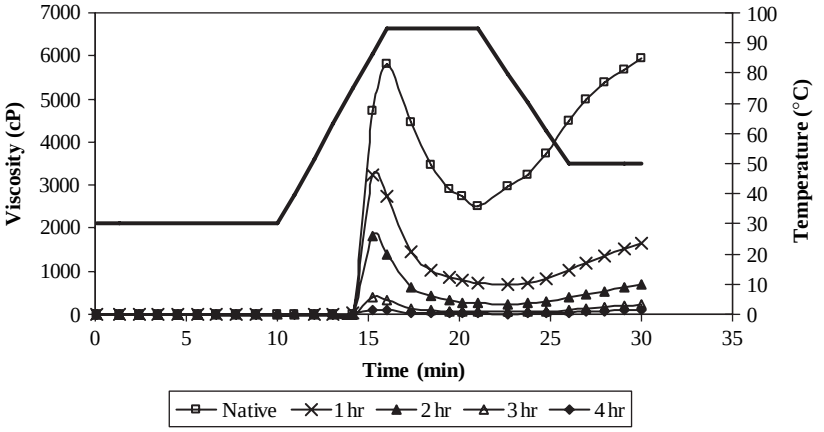


Figure 1 Pasting curves of native and acid hydrolysed corn starch samples.

Table 2 Pasting properties of resistant starch preparations.

Hydrolysis time (hr)	Storage period at 95 °C (day)	Peak viscosity (cP)	Breakdown (cP)	Final viscosity (cP)
0	0	9986 a	3711 a	10345 a
	2	3680 c	238 b	8599 b
	3	4040 b	304 b	8816 b
	4	3668 c	443 b	7716 c
0.5	0	3025 a	920 a	4773 a
	2	1397 bc	153 b	3079 c
	3	1281 c	66 b	2693 d
	4	1500 b	101 b	3432 b
1.0	0	1744 a	589 a	2636 a
	2	1080 b	104 b	2140 b
	3	808 c	89 b	1704 c
	4	845 bc	85 b	1670 c
1.5	0	1551 a	638 a	2027 a
	2	401 b	41 b	867 c
	3	417 b	40 b	923 b
	4	358 c	32 b	799 d
2.0	0	1152 a	294 a	1454 a
	2	288 b	24 b	651 b
	3	266 b	21 b	622 b
	4	282 b	18 b	646 b
2.5	0	477 a	71 a	714 a
	2	303 b	15 b	566 c
	3	254 c	17 b	533 d
	4	312 b	14 b	619 b
3.0	0	218 a	45 a	353 a
	2	126 b	14 b	257 b
	3	105 b	12 b	236 b
	4	129 b	12 b	277 b
3.5	0	195 a	46 a	310 a
	2	106 b	9 b	224 b
	3	69 d	10 b	149 b
	4	88 c	10 b	210 b
4.0	0	90 a	21 a	146 a
	2	59 ab	15 a	113 a
	3	49 b	10 a	93 a
	4	32 b	12 a	113 a

Means with the same letter within each hydrolysis level are not significantly different ($p < 0.05$).

viscosity values of the 0, 2, 3, and 4 days stored samples produced from 4 h hydrolysed sample were not significantly different. On the other hand, there was generally no regular effect of storage period (2, 3, and 4 days) at 95°C on the peak and final viscosity values of RS preparations within each hydrolysis level. However, the difference in breakdown viscosity values of 2, 3, and 4 days stored samples were not significant ($p < 0.05$).

Since the starch is gelatinized prior to RS formation, it is expected to observe a viscosity value higher than "0" at the initial stage of RVA curve (before heating). This is referred to as cold peak viscosity in the related literature.[29] However, in the present study, such cold peak viscosity values were not observed in the RVA curves (Figure 2) and the viscosity values increased as the temperature increased. These results are in

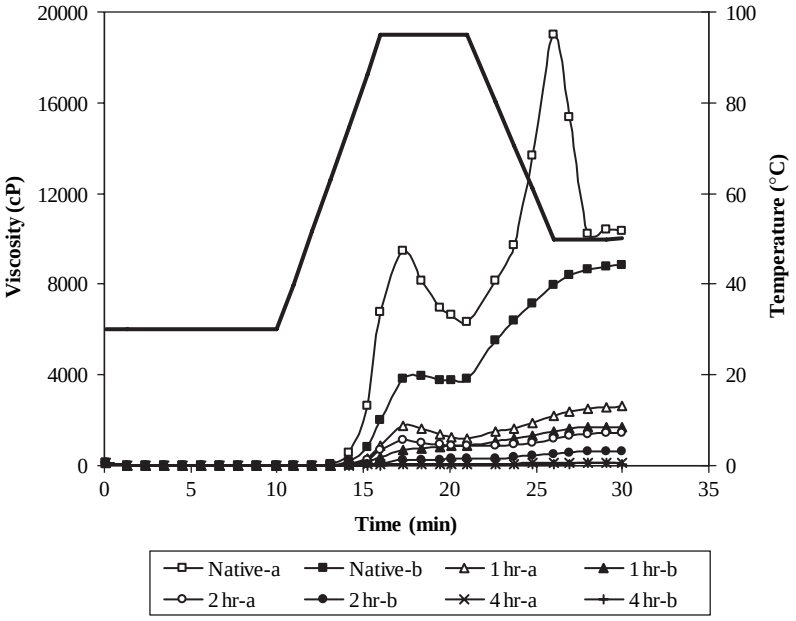


Figure 2 The pasting curves of resistant starch preparations obtained from native and acid hydrolysed starches (hydrolysis time: 1, 2, 4 hr). a: Unstored sample; and b: sample stored for 3 days.

agreement with those of Becker et al.[30] The lack of cold peak viscosity might probably be due to loose rearrangement of starch chains with a few (small number of) H-bonds. This can be different from regular retrogradation of starch during RS formation. The loose rearrangement of starch chains might prevent water uptake at the initial stages of RVA test. Later on, viscosity increased as a result of water uptake by the system following H-bond disruption due to heating. Then a peak is formed. Although, the reason for the formation of this peak is different, its shape is similar to the one observed during starch gelatinization.

Resistant Starch Content

Resistant starch contents of the samples are presented in Figure 3. While native and acid hydrolysed starch samples did not contain any RS (data not presented in Figure 3), the RS contents increased to 13.6–16.7% as a result of storage at 95°C for different periods after autoclaving. For each storage period (2, 3, and 4 days), the resistant starch content increased as the hydrolyzation degree increased up to 2.5 h hydrolyzation level. Above this level, RS contents decreased. On the other hand, as indicated by overlapping error bars, there was no significant difference between the RS contents of the samples hydrolysed at the same level but stored at different periods (2, 3, and 4 days) at 95°C after autoclaving.

RS contents of the samples dried without storage increased from 8.1 to 13.9% as the hydrolyzation degree increased. RS contents of these samples were lower than those of the stored samples (2, 3, and 4 days) at all hydrolysis levels up to 3 h. However, RS contents of the samples dried without storage and those of the stored samples were comparable for 3.5 and 4.0 h hydrolysed samples. It has been also reported in earlier literature that

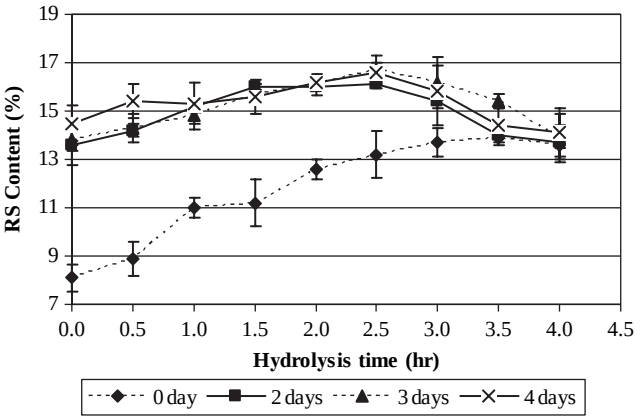


Figure 3 Resistant starch contents of the acid hydrolysed samples autoclaved and stored at 95°C for different periods.

acid-pretreated starch produced higher RS values than the native starch which might be attributed to increased rate of retrogradation in acid-hydrolysed starch.[31] Partial acid hydrolysis produces short linear chains enhancing the mobility of the molecules. The shorter linear chains appear to participate in the formation of resistant portions through rearrangement and recrystallization of starch during autoclaving and cooling.[32] In the present study, there are two major factors affecting RS formation: the molecular size (hydrolysis time) and storage time after autoclaving. Molecular size influences RS formation by affecting the mobility of starch chains. For the stored samples, because of the availability of adequate time for RS formation, the molecular size of the samples plays the major role in RS formation. Optimum RS content was achieved at 2.5 h hydrolysis time, and above this level, the RS contents decreased due to excessive hydrolyzation. In the samples prepared without storage, RS formation occurs only during drying. Although, at each hydrolysis time the stored and unstored samples have the same average molecular size, the time during drying of unstored samples is not enough for achieving (high) RS contents comparable to those of the stored ones. So, a steady increase in RS content was observed in the samples prepared without storage, as the hydrolysis time increased (upto 3.5 h hydrolysis). But the RS contents were still lower than those of the stored samples.

Functional Properties

Solubility, water binding capacity, emulsion capacity, and stability of the native and acid hydrolysed starch samples and RS preparations are shown in Figure 4. While the solubility values of the native and acid hydrolysed samples were less than 1%, the solubility values of the RS preparations were higher and increased up to 5.2% with increasing hydrolysis time and storage at 95°C (Figure 4a). Water binding values of the native and acid hydrolysed samples were low and the highest water binding value was obtained for the 4.0 hr acid hydrolysed sample (120.3%). However, the water binding values of RS preparations were much higher (at least two-fold) than those of the acid hydrolysed samples. The increase in water binding value is mainly due to the gelatinization caused by heating and autoclaving. RS formation does not play a major role in this respect. It is thought that these products could be used in food formulations in which relatively higher

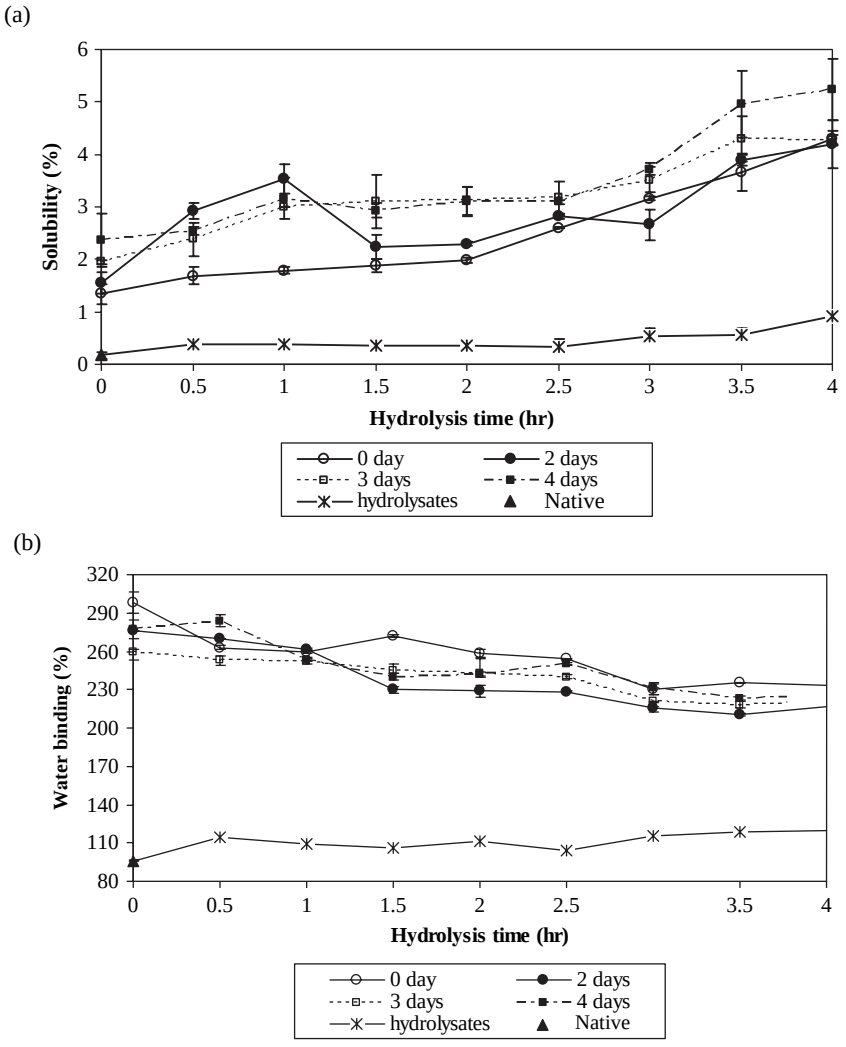


Figure 4 Functional properties of the native and acid hydrolysed starch samples and resistant starch preparations produced by autoclaving and storage at 95°C for different periods. a) Solubility; b) water binding; c) emulsion capacity; and d) emulsion stability.

water binding properties are desired as compared to native starch. As the hydrolysis time increased, water binding values of RS preparations generally decreased (Figure 4b).

There are some studies investigating the solubility and water binding capacity values of different starches. Singh et al.[17] found that pea starch had water binding capacity of 0.13–4.15 g/g and solubility of 12.5%. Aparicio-Saguilan et al.[15] reported that solubility of native banana starch increased with increasing temperature. Autoclaved samples presented lower solubility than corresponding raw materials (native and lintnerized samples). Sandhu and Sing[16] reported that solubility and water binding capacity values of starches from different corn lines were in the range of 15.3–22.4% and 77.6–88.5%, respectively. The water binding capacity results of the present study were comparable with those of Sandhu and Sing[16] but the solubility values determined in the present study

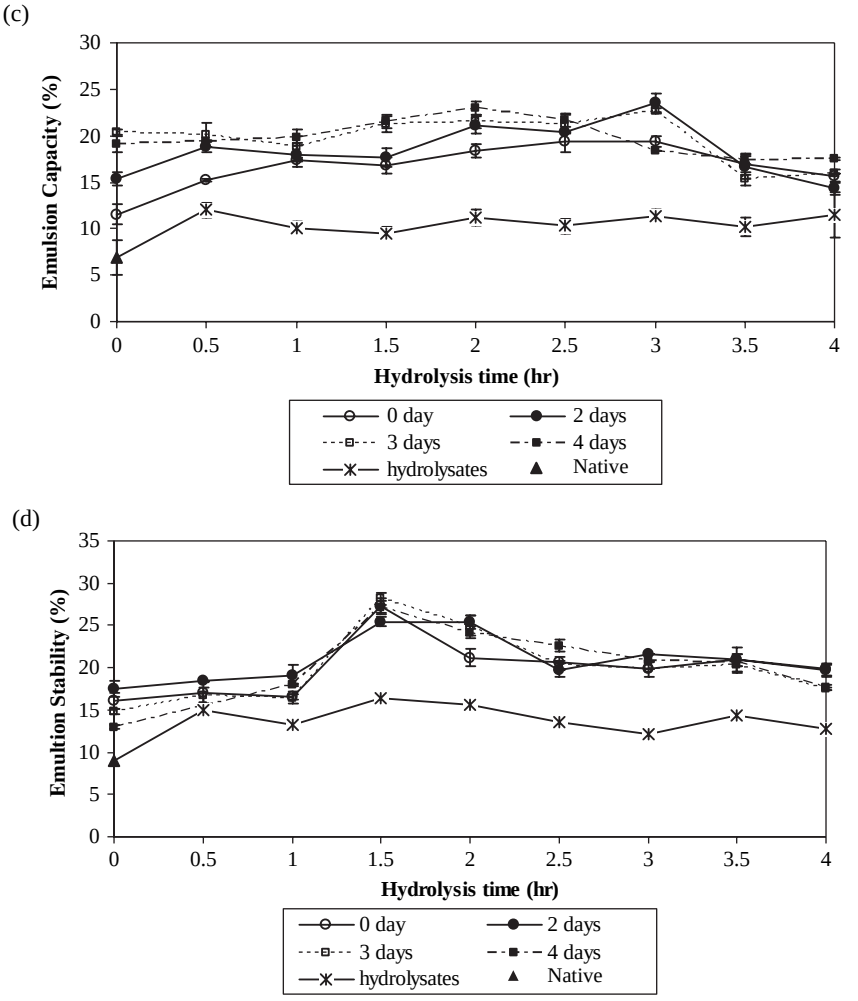


Figure 4 (Continued).

were lower. The difference might be due to the variations in method parameters (e.g., temperature).

Proteins are commonly used as emulsion forming and stabilizing agents. On the other hand, starch can not produce emulsion by itself, but might effect emulsion properties. Therefore, in the present study, effects of various starch preparations on the emulsifying properties of soy protein solutions were investigated. Emulsion capacity and emulsion stability values of soy protein solution (0.05%) was found to be 22% and 18%, respectively. Emulsion capacity and stability values of soy protein solution supplemented with the acid modified starch samples were lower than those of the soy protein solution on its own. The results indicated that the native and acid modified starch samples affected the emulsion properties of the soy protein inversely. On the other hand, RS preparations did not have a deteriorating effect on the emulsion capacity values of the soy protein solution. Emulsion capacity of soy protein solution supplemented with RS containing samples

prepared from 3.5 and 4.0 hr hydrolysed samples (stored at 95°C for 2, 3, and 4 days) were lower than those of the other samples (Figure 4c). Emulsion stabilities of soy protein solutions supplemented with RS preparations were within the range of 13.0–28.2%. Most of them were higher than that of soy protein solution. An increase in emulsion stability value was observed for the starch samples with low hydrolyzation levels (up to 1.5 h) and then they gradually decreased (Figure 4d). Although the effects of RS preparations on emulsion properties of soy protein were better than those of the hydrolysates, the RS preparations do not seem to have an improving effect on emulsion properties of soy protein. RS formation does not seem to affect fat binding properties of respective hydrolysates to a considerable extent. All of the RS preparations as well as the hydrolysates were found to have similar fat binding values of 85 ± 10mL/100g.

CONCLUSIONS

The results showed that the RS contents of acid-modified corn starch samples increased up to 16.7% after autoclaving and storage. RS preparations did not have a deteriorative effect on emulsion properties of soy proteins. Heating and autoclaving treatments caused an improving effect on the solubility and water binding properties. The RS preparations obtained in the present study seem to be suitable for the food products, which require relatively higher water binding properties. Further studies are needed to produce resistant starch preparations with improved functional properties.

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