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Evaluation of Molecular Weight Distribution, Pasting and Functional Properties, and Enzyme Resistant Starch Content of Acid-modified Corn Starches

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Abstract: The aim of this study was to produce resistant starch preparations from acid-modified corn starches prepared at various hydrolysis levels (0.5-4.0 hr). Effect of autoclaving cycles on resistant starch (RS) formation was investigated. Molecular weight distribution, pasting and functional properties of acid-modified corn starches were determined. For RS formation native and acid-modified starch samples were gelatinized and autoclaved (1 or 2 cycles). While native and acid-modified starches did not contain any RS, the levels increased to 9.0-13.5% as a result of storage at 95°C after first autoclaving cycle. Second autoclaving cycle together with storage at 95°C brought final RS contents of the samples incubated at 4 and 95°C after the first cycle to comparable level. As acid modification level increased, the amount of high molecular weight fractions decreased, resulting in significant decreases in viscosities ($p<0.05$). The samples produced in this study had low emulsion stability and capacity values.

Keywords: acid modification, resistant starch, autoclaving cycle, emulsifying property, pasting property

Introduction

A part of the ingested starch is not digested in the small intestine of healthy humans. This is called resistant starch (RS). Due to its similar physiological properties, it is generally considered as a constituent of dietary fiber. The physiological importance of RS was investigated in several studies, mainly with regard to the formation of short-chain fatty acids (SCFAs) via fermentation in the colon. SCFAs help to prevent colorectal cancer (1). Furthermore, slow digestion of RS reduces postprandial glycemia and insulinemia and has the potential for increasing the period of satiety (2). RS has also been shown to be effective in lowering plasma cholesterol levels in genetically obese and lean rats (3) and humans (4,5). Resistant starch is also reported to enhance the ileal absorption of a number of minerals in rats and humans (6,7).

RS contents of foods generally range between 0-4%. A higher amount of RS in food is recommended due to its preventative and therapeutical health effects (8). RS can be naturally present in foods or may be formed due to the processing conditions (9). Resistant starches have been assigned to 4 categories: physically inaccessible starch locked within cell walls (RS_1), granular starch (RS_2), retrograded or crystalline nongranules (RS_3), and chemically modified starches (RS_4) (10-12). RS_3 is the most common in the human diet because it is formed mainly as a result of food processing (13). The RS_3 content of the foods is generally low. RS_3 levels up to 3% have been reported in baked foods, pasta products, and processed cereals and tubers. The levels can be increased by heating and cooling cycles (14). Formation of RS_3 can be considered as a crystallization process of amylose. Many factors may influence the crystallization process, and thus the formation of RS_3, such as amylose content and chain length, autoclaving temperature, storage time, and temperature of the starch gels (15). Acid modification is widely used in the starch industry to prepare thin boiling starches for use in food, paper, textile, and other industries. Hydrolysis level leads to changes in various properties of starch. The retrogradation rate of acid-thinned starch gels was reported to increase as hydrolysis proceeded. Acid modification also increased solubility and gel strength and decreased viscosity of starches (16-18).

Besides their nutritional value, starches have been used for their functional properties. There are some studies investigating the swelling, solubility, water binding capacity values (19-21), and emulsion properties (22,23) of various starches. However, to the best of our knowledge, there are no studies investigating the effects of acid-modified starches on emulsion properties. The aim of this study was to produce resistant starch preparations from acid-modified corn starches at various hydrolysis levels by autoclaving and cooling cycles. Molecular weight distribution, pasting and functional properties of acid-modified starches were also determined.

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Materials and Methods

Material 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 hr). After the incubation period, the pH of the suspension was adjusted to 6 with 0.25 M NaOH. Then the samples were washed 3 times with distilled water and centrifuged (Heraus Labofuge, Hanau, Germany) at 150×g for 5 min (24). 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 method (25).

Molecular weight distribution Gel filtration technique was used for the determination of molecular weight distribution (26,27). As a mobile phase, 0.1 M KOH was used at a flow rate of 0.5 mL/min. Starch samples dissolved in 0.5 M KOH (50 mg/5 mL) were injected to Sepharose CL-6B (Amersham Biosciences, Uppsala, Sweden) column (1.5×75 cm) and 1.5 mL fractions were collected (Retriever 500; Isco Co., Lincoln, NE, USA). The dextrans with molecular masses of 10, 70, 110, 500, and 2,000 kDa were used as molecular weight markers (Amersham Biosciences). Total carbohydrate contents of the fractions were determined by using phenol sulfuric acid method (28).

Pasting properties Pasting properties of the hydrolysates were tested using a rapid visco-analyzer (RVA 4; Newport Scientific, Warriewood, Australia). Four g sample of native or hydrolyzed corn starch and 25 g distilled water were placed in an aluminum sample canister. The RVA pasting curve was obtained by using a profile recommended for the acid-modified starch by the Newport Scientific. The RVA pasting curve was obtained by using the 20 min test profile; initial equilibrium at 30°C for 30 sec, heating to 95°C over 4 min, holding at 95°C for 4 min, cooling to 30°C over 5 min, and holding at 30°C for 6.5 min. The peak viscosity, Visc-9 (viscosity at 9 min) and final viscosity values were evaluated with data analysis software (Thermocline for Windows, Newport Scientific). The results are reported as means of duplicate analyses.

Functional properties Solubility values of the samples were determined using a method based on Singh and Singh (29). A 0.5 g of sample was added to 5 mL distilled water and vortexed for 15 sec every 5 min. After 40 min it was centrifuged (Heraus Labofuge) 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;

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

For determination of fat binding capacity a 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 at 2,100×g for 10 min. The unbound oil was separated by using a pipette and the volume was measured. Fat binding capacity was expressed as the amount (mL) of oil bound by a 100 g sample (30).

Emulsion capacity and stability were determined according to Ahmedna *et al.* (31) and samples were prepared according to Abdul-Hamid and Luan (32). 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; Art Labortechnik, 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 emulsion capacity (%). For the determination of emulsion stability, homogenized sample was incubated at 45°C for 30 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 emulsion stability (%). The results on all of the functional properties are reported as means of triplicate analyses.

Resistant starch formation and determination For resistant starch formation native and some of the acid-modified starch samples (1 and 2 hr) were used. The samples were suspended in water and gelatinized at 80°C for 10 min, autoclaved (1 or 2 cycles) at 121°C for 30 min. For the first set of samples, starch gels were stored at 2 different temperatures (4 and 95°C) for various storage periods (1, 2, and 5 days) after the autoclaving. For preparing another set of samples, the autoclaved samples were stored at 2 different temperatures (4 and 95°C) for 24 hr and subjected to the second autoclaving cycle followed by storing at 95°C for various storage periods (1, 2, and 5 days). Both set of samples were dried at 50°C after the storage. The dried samples were ground and RS contents were determined by the enzymatic-gravimetric procedure (33). Sequential enzymatic digestion was applied to the samples by using heat stable α-amylase, protease, and amyloglucosidase to remove digestible starch and protein. Enzyme digest was treated with alcohol before filtering, and RS residue was washed with alcohol and acetone, dried, and weighed. The results are reported as means of triplicate analyses.

Statistical analysis Data were analyzed for variance using the MSTAT-C statistical package. When significant ($p<0.05$) differences were found, the least significant difference (LSD) test was used to determine the differences among means (34).

Results and Discussion

Molecular weight distribution Gel filtration chromatograms of native and acid-modified starch samples are shown in Fig. 1a and 1b. Gel filtration chromatograms were separated into 3 areas (low-, medium-, and high-molecular weight fractions) to compare the molecular weight distributions of the samples and the relative quantities (%) of these fractions are shown in Table 1. Gel filtration results indicated that the amount of high molecular weight fractions decreased as the hydrolysis time increased. This decrease resulted in increases in medium molecular weight fractions at lower hydrolysis times (up to 2.5 hr hydrolysis). The increase in the amount of medium molecular weight

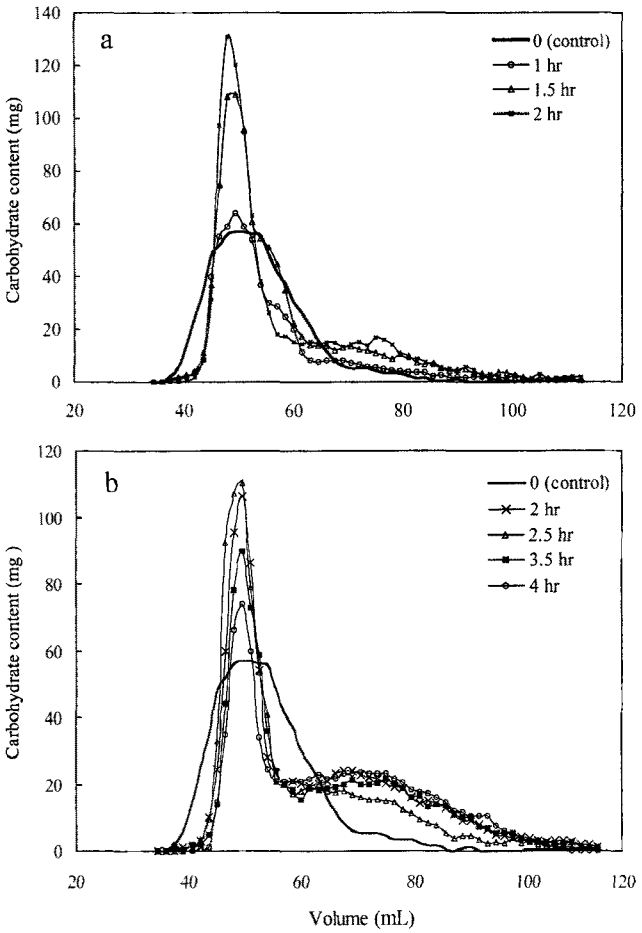

Fig. 1. Gel filtration chromatograms of native (a) and acid-modified (b) corn starch samples.

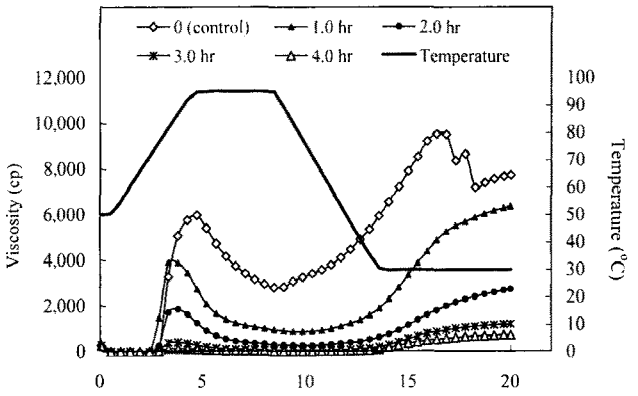

Fig. 2. Rapid visco-analyzer curves for native and selected acid-modified corn starch samples hydrolyzed for various periods.

fractions at the lower hydrolysis times is probably due to the limited hydrolysis of the high molecular weight fractions. Longer hydrolysis times caused further decreases in the molecular weight and the amount of medium molecular weight fraction decreased as well. The changes in these 2 fractions caused an increase in the amount of low molecular weight fractions.

Pasting properties RVA pasting curves of native and selected acid-modified starch samples are shown in Fig. 2 and their pasting properties are presented in Table 2. All of the viscosity values of acid-modified starch samples were found to be less than those for the native starch. As the level of acid modification increased, all of the RVA viscosity values of the hydrolysates decreased progressively and significantly ($p<0.05$). The major effect of acid on starch molecules is the reduction of the molecular weight and the decreases in the viscosity values might be due to reduced molecular weight (35).

Functional properties Solubility, water binding and fat binding capacities, and emulsifying properties of the native and some of the acid-modified corn starch samples are presented in Table 3. Effects of acid modification on these properties were not substantial. Therefore, the data for 1, 2, 3, and 4 hr hydrolyzed samples were given to show the general trend. The solubility values of the acid-modified corn starches increased significantly with increasing hydrolysis time in accordance with the decrease in the molecular weight. However, the increase in solubility was not considerable enough to create substantial changes in food systems. Water binding values of the samples increased significantly. Higher water binding values were obtained for the samples hydrolyzed for 3 and 4 hr. The acid-modified corn starches were found to have similar fat binding values of 87±3 mL/100 g ($p>0.05$).

There are some studies investigating the solubility and water binding capacity values of different starches. Singh *et al.* (21) found that pea starch had water binding capacity of 0.13-4.15 g/g starch and solubility of 12.5%. Sandhu and Singh (20) 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

Table 1. Relative quantities of high, medium, and low molecular weight fractions of native and acid-modified starch samples

Hydrolysis time (hr)	High Mw fractions (%) (>2,400 kDa)	Medium Mw fractions (%) (2,400-710 kDa)	Low Mw fractions (%) (<710 kDa)
0 (control)	14.0	57.0	29.0
1.0	5.9	64.2	29.9
1.5	4.1	62.5	33.4
2.0	2.8	63.6	33.6
2.5	3.3	59.9	36.8
3.0	2.9	49.3	47.8
3.5	1.7	48.3	50.0
4.0	1.1	39.9	59.0

Table 2. Pasting properties of native and acid-modified corn starch samples

Hydrolysis time (hr)	Peak viscosity (cp)	Visc-9[1] (cp)	Final viscosity (cp)
0 (control)	6,003a[2]	2,907a	7,749a
0.5	4,538b	1,260b	7,670a
1.0	4,009c	905c	6,367b
1.5	2,528d	451d	3,592c
2.0	1,879e	284e	2,744d
2.5	1,196f	146f	2,008e
3.0	428g	60g	1,221f
3.5	390g	61g	1,127f
4.0	133h	26g	733f
LSD (p<0.05)	120.0	68.8	510.2

[1]Viscosity at 9 min.
[2]Means with different letters within each column are significantly different (p<0.05).

Table 3. Functional properties of native and acid-modified corn starch samples

Hydrolysis time (hr)	Solubility (%)	Water binding (%)	Fat binding (mL/100 g)	Emulsion capacity (%)	Emulsion stability (%)
0 (control)	0.17c[1]	95.7d	89.3	5.5b	8.9
1.0	0.37bc	108.8c	89.3	10.0a	10.9
2.0	0.36bc	110.7bc	84.5	11.2a	13.5
3.0	0.54b	115.7ab	84.4	11.4a	13.5
4.0	0.91a	120.3a	84.5	11.6a	12.8
LSD (p<0.05)	0.215	5.06	NS[2]	3.31	NS

[1]Means with different letters within each column are significantly different (p<0.05).
[2]Not significant.

water binding results of the present study were comparable with those of Sandhu and Singh (20) but the solubility values determined in the present study 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 affect emulsion properties (23). Therefore, in the present study, effects of various starch samples on the emulsifying properties of soy protein solutions were investigated. Emulsion capacity and emulsion stability values of soy protein solution (0.05%) were found to be 22 and 18%, respectively. Emulsion capacity and stability values of soy protein solution supplemented with the native and acid-modified starch samples were substantially 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. Much higher values are reported for good emulsions in the literature (36). The emulsion capacities of soy protein solution supplemented with the hydrolysates were significantly higher than that of the soy protein solution supplemented with native corn starch. Native and acid-modified starches are in granular form and do not have capacity for remaining at oil–water interface (23) and precipitated upon centrifugation applied after emulsion formation during the determination of emulsion capacity and stability values. In the present study, the properties of the emulsion formed by the soy protein deteriorate probably due to precipitation of starch granules.

Resistant starch content In a previous study on optimization of the processing parameters (hydrolysis time, storage time, and temperature) of enzyme resistant starch formation, 0, 1, 2, 3, and 4 hr hydrolysis times were tested and it was found that resistant starch content decreased for the samples hydrolyzed for 3 and 4 hr (37). Therefore, in this study 1 and 2 hr hydrolyzed samples were used for RS formation. RS contents of the samples produced by 1 and 2 autoclaving cycles are presented in Fig. 3.

While native and acid-modified starches did not contain any RS, the levels increased to 9.0-13.5% as a result of storage at 95°C after the first autoclaving cycle (Fig. 3). However, storage of autoclaved starch samples at 4°C increased RS level to a lower extent. Furthermore, the RS contents of the autoclaved and stored (at 4°C) acid-modified starches generally increased with increasing acid modification level. RS contents increased as the storage time increased for the native and 1 hr hydrolyzed starch samples stored at 95°C after the first autoclaving cycle.

Following the second autoclaving cycle and storage, RS contents of the samples kept at 4°C after the first autoclaving cycle substantially increased while no considerable difference was observed in the RS contents of the ones stored at 95°C after the first cycle. In other words, the second autoclaving cycle together with the storage at 95°C brought the final RS contents of both set of samples (the ones incubated at 4°C and the ones incubated at 95°C after the first cycle) to a comparable level. Hence the number of autoclaving cycles and the storage temeperature of the starch gels must be selected according to RS contents of the samples.

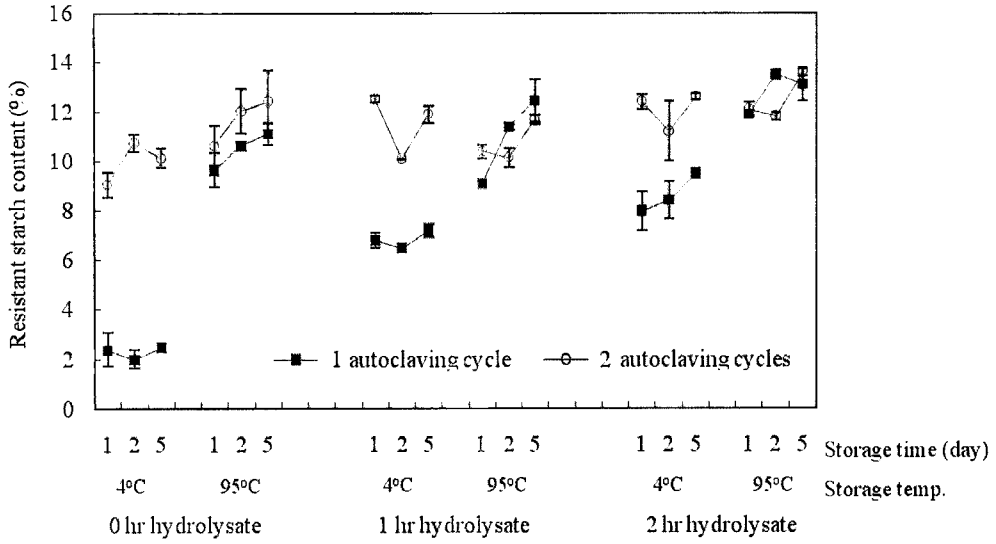

Fig. 3. Resistant starch contents of the corn starch samples produced by 1 and 2 autoclaving cycles.

In conclusion, the main effect of acid hydrolysis on starch molecules was the reduction of the molecular weight as determined by gel filtration. As the level of acid modification increased, the viscosity values of the samples decreased probably due to reduced molecular weight. While native and acid-modified starches did not contain any RS, the levels increased to 9.0-13.5% as a result of storage at 95°C after the first autoclaving cycle. The second autoclaving cycle together with the storage at 95°C brought the final RS contents of the samples incubated at 4 and 95°C after the first cycle to a comparable level. Increased RS content is expected to have various health benefits such as protecting against colorectal cancer, lowering plasma cholesterol and improving glucose tolerance. Further studies are needed to extend the knowledge on RS production and improve the functional properties and applications of starch preparations in various food systems.

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