

Influence of Botanical Source and Processing on Formation of Resistant Starch Type III

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ABSTRACT

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Influence of botanical source and gelatinization procedure (autoclaving or boiling) on resistant starch (RS) formation was investigated in starches from wheat, corn, rice, and potato. RS yields did not vary within the same sample but differed among samples with different starch botanical sources. Differences also existed in RS contents in native and retrograded starches. Slight or minor variations in RS values were found after

both gelatinization procedures, although no clear pattern was found in the behavior of samples based on gelatinization procedure. The degree of polymerization (DP) of retrograded samples was assigned using high-performance anion exchange chromatography with pulsed amperometric detector (average DP 50–60), with no differences between autoclaved and boiled samples.

From a physiological perspective, not all starch forms behave in the same way. Resistant starch (RS) is a fraction of starch that is not digested in the human small intestine, although it is partially fermented in the large bowel (Asp 1992). Raw starches and coarsely ground grains are partially inaccessible to digestive enzymes due to partial crystallinity or incomplete milling. Some processing techniques and storage methods have a marked influence on the levels of starch resistant to digestion (Muir and O'Dea 1992). Retrograded starch, also termed RS type III, is the most common in the human diet and, from a technological point of view, is the most important type, because it is formed mainly as a result of food processing.

Formation of RS type III is achieved by gelatinization and retrogradation of starch. During the gelatinization process, the molecular order of the starch granule is gradually destroyed, and the starch becomes easily digestible. When cooled, the gel forms a partially crystalline structure: retrograded starch. RS type III is formed during two different gelatinization processes: autoclaving and cooking at atmospheric pressure. Different RS yields have been obtained (Björck et al 1987; Berry et al 1988; Siljeström et al 1989, 1990).

The aim of this work was to compare the influence of both gelatinization procedures and different botanical sources on formation of RS type III and investigate the potential relationship between RS levels in raw material and RS type III level in retrograded starch obtained from gelatinization processes.

MATERIALS AND METHODS

Native wheat, corn, potato, and rice starches were studied. All were purchased from Sigma Chemical Co. (St. Louis, MO).

Gelatinization

High pressure process. An autoclave (Berthod, GmbH, Eningen, Germany) was equipped with a pressure glass with vacuum line and thermocouple, heating cover with magnetic stirrer, thermosensor, and temperature and stirring rate control system.

The sample (5.0 g) was dispersed in 40 mL of distilled water. Gelatinization conditions were standardized by Escarpa et al (1996). Pressure was set at 2 bar of N₂. Stirring speed was 1,300 rpm, gelatinization temperature was 120°C, and process time was 32 min.

Cooking process. Sample (5.0 g) was weighed in a centrifuge tube and mixed with 40 mL of distilled water. A stirring bar was placed in the tube. Capped tubes were submerged in a boiling water bath and boiled and stirred for 45 min.

Retrogradation

Starch suspensions were poured into a petri plate, cooled to room temperature, and frozen at –20°C. After 16 hr, samples were defrosted at room temperature for 8 hr and dried at 60°C in an air-circulating oven at a flow rate of 2.3 m³/min for 16 hr. Finally, samples were milled to a particle size of 1 mm with a sample mill (Cyclotec 1093, Tecator, Höganäs, Sweden).

Isolation of RS

Tris-maleate buffer (9 mL) at pH 6.9 and 1 mL of a solution containing 40 mg of α -amylase (type VI from porcine pancreas, A-3176, Sigma) were added to retrograded starch samples (100 mg). The mixture was incubated at 37°C for 16 hr in a shaken water bath. Centrifugation (2,500 \times g, 15 min) of samples was performed to remove supernatant containing digested starch. Residues were washed with distilled water and centrifuged twice. Finally, RS residues were frozen and lyophilized (Lyophilizator, Virtis, New York). Residues were milled to <1 mm.

RS Determination

RS was measured using the procedure of Goñi et al (1996). The method has the following steps: removal of protein with pepsin (7190, Merck, Darmstadt, Germany, 40°C, 1 hr, pH 1.5), incubation with α -amylase (A-3176, 37°C, 16 hr) to hydrolyze digestible starch, treatment of precipitates with 2M KOH to solubilize RS, incubation with amyloglucosidase (102857, Boehringer GmbH, Mannheim, Germany, 60°C, 45 min, pH 4.75), and determination of glucose, using the glucose oxidase assay (676543, Boehringer). RS was calculated as glucose \times 0.9.

High-Performance Anion Exchange Chromatography with Pulsed Amperometric Detector

High-performance anion exchange chromatography with pulsed amperometric detector (HPAEC-PAD) was used to analyze the degree of polymerization (DP) of RS samples. NaOH (1.5M, 250 μ L) was added to 10 mg of purified RS sample while mixed. The mixture was stirred overnight in a cold room (6°C). Just before the run, 2.25 mL of Milli-Q water was added to each sample. Samples were filtered through 0.2- μ m mesh nylon filters, and 25 μ L of the solution was subjected to HPAEC (DX500, Dionex Corp., Sunnyvale, CA) equipped with a Dionex CarboPac PA-100 column (4 mm \times 250 mm) in combination with a CarboPac PA-100 guard column and run at 25°C. Eluents were A: Milli-Q water, B: 150 mM NaOH, C: 150 mM NaOH and 500 mM sodium acetate, and D: 500 mM NaOH. Elution (1 mL/min) was done using the gradient reported

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in Table I. Solvents were prepared from a 50% NaOH solution (7067, JT Baker, Deventer, the Netherlands) with Milli-Q water, filtered through Millipore filters (0.22- μ m mesh), degassed, and stored under helium (4 psi). The eluent was monitored with an electrochemical detector (ED40, Dionex) in the pulsed amperometric detection mode. A reference Ag/Ag Cl electrode with a working gold electrode was used with the following pulse potentials and durations: $E_1 = 0.05$, 200 msec; $E_2 = 0.75$, 200 msec; and $E_3 = -0.15$, 400 msec. Instrument control and data collection were performed using chromatography work station software (Peak Net, Dionex, Sunnyvale, CA). DP was assigned by spiking RS samples with maltoheptaose (DP 7).

Statistics

Results are expressed as mean \pm standard deviation. Comparison of means was performed by one-way analysis of variance (ANOVA) (Statgraphics, version 5.1, SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

RS Yields

Analysis of RS was performed in native starches, as well as in retrograded starches after gelatinization in the autoclave (high pressure) or boiling water bath (atmospheric pressure). Data are reported in Table II. Native starch forms differed in RS yields. As expected, potato had the highest value (79.48% db) due to its granule structure. Rice and corn had the same RS values (28.9 and 28.93% db), and wheat had the lowest value among cereals (14.6% db).

The significant differences ($P \leq 0.05$) in RS yields between native and retrograded starches can be appreciated, and there is a clear decrease in RS content, except in wheat. The values obtained after both gelatinization procedures were slightly higher in the case of wheat and corn when autoclaved. RS yields were almost the same for both procedures with potato, whereas analysis of rice starch produced a higher value after boiling than after autoclaving. Therefore, there is no clear pattern in the behavior of the samples due to gelatinization procedure.

The range in RS yields did not vary within the same sample but did differ among starch with different botanical sources. Rice had the smallest amount of RS when it was retrograded (3.22–4.52%),

whereas its native RS content was close to that of native corn (28.9 and 28.93%, respectively). Potato starch had the highest values, both when native and retrograded compared to the other starches. In general, cereals had lower RS values than tubers.

Escarpa et al (1996) reported similar RS yields after autoclaving but lower RS values after boiling; their results, however, were drawn from only one sample (potato) and under various boiling conditions. In general, it is difficult to compare the current results with values reported in the literature (Björck et al 1987; Berry et al 1988; Siljeström et al 1989, 1990), because sample origin, preparation of sample, and procedure used to measure RS yields were not always the same. The slight variations in final values maybe due to the factors that play a part in the formation process. Taking these factors into account, the trend is similar in all cases: higher levels of RS in native than in retrograded forms, with differences between cereal and tuber starches. No clear relationship was found between RS levels in raw materials and formation of RS type III.

HPAEC-PAD

The DP of RS isolated from retrograded samples was analyzed using HPAEC, which has been used successfully to obtain chain-length distribution information to DP 50–60 and higher values when a change in gradient conditions was included (Gidley et al 1995).

Typical chromatograms for wheat (Fig. 1A and B), corn, rice, and potato RS, gelatinized using both methods, were obtained. As Gidley et al (1995) pointed out, the sharp nature of the peaks could confirm that within the fractionated range, samples were mainly linear, because branching leads to irregular peaks, and less resolution. Once DP 7 (maltoheptaose) was established in the standards assay, the remaining peaks were assigned by “spiking” the samples with this peak, and peaks with increasing elution time to increasing DP values were assigned sequentially. Good resolution was found up to DP 50–60, with at

TABLE I
Elution Gradient (B, C, D) for High-Performance Anion Exchange Chromatography^a

Time	Flow	B (%)	C (%)	D (%)
Initiation	1.00	20.0	80.0	0.0
0.00	1.00	80.0	20.0	0.0
12.00	1.00	80.0	20.0	0.0
13.00	1.00	80.0	20.0	0.0
93.00	1.00	18.0	82.0	0.0
93.10	1.00	0.0	100.0	0.0
94.00	1.00	0.0	100.0	0.0
95.00	1.00	0.0	0.0	100.0
100.00	1.00	0.0	0.0	100.0
101.00	1.00	20.0	80.0	0.0

^a B: 150 mM NaOH; C: 150 mM NaOH, 500 mM sodium acetate; and D: 500 mM NaOH

TABLE II
Resistant Starch (mean \pm SD, db) for Native and Retrograded Wheat, Corn, Rice, and Potato Starches^a

Starch Source	Native	Gelatinized at 120°C	Gelatinized at 100°C
Wheat	14.61 \pm 2.5 b	13.41 \pm 0.4 b	10.37 \pm 0.2 a
Corn	28.93 \pm 2.1 c	10.55 \pm 0.3 b	9.55 \pm 0.7 a
Rice	28.90 \pm 3.2 c	3.22 \pm 0.1 a	4.52 \pm 0.7 b
Potato	79.48 \pm 3.1 b	16.73 \pm 0.9 a	17.66 \pm 1.1 a

^a Different letters in each row indicate significant differences at $P \leq 0.05$. $n = 4$.

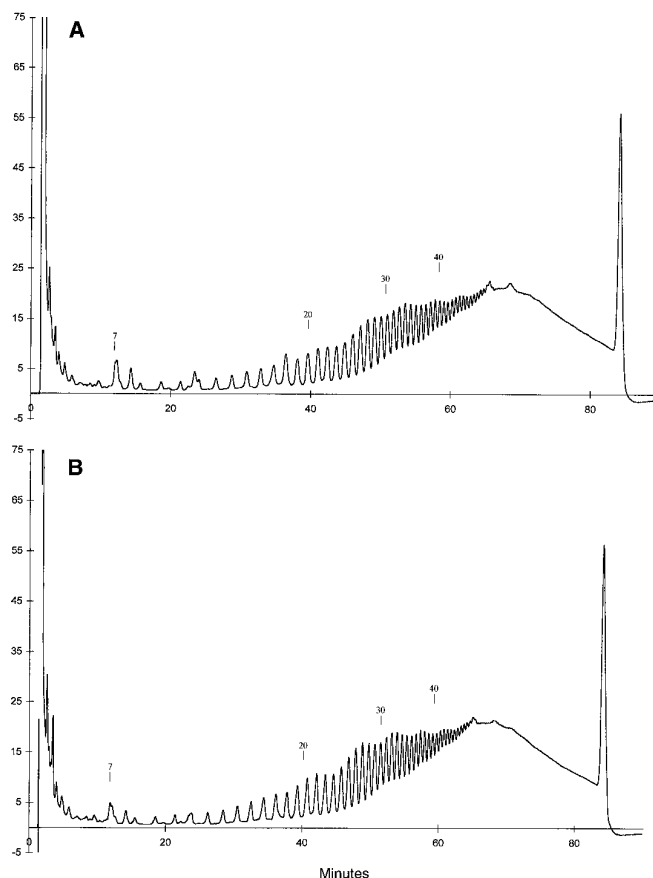


Fig. 1. High-performance anion exchange chromatography profile of resistant starch in wheat starch gelatinized by autoclaving (A) and boiling water bath (B).

least partial peak resolution up to DP 70–80. Chain lengths with peak values of DP 50 (Russell et al 1989) and 65 (Siljeström et al 1989) have been found in RS formed in autoclaved wheat starch gels, which led to the conclusion that RS consisted of crystallized linear α -glucans of relatively low molecular weight, with only minor amounts of high molecular weight material remaining. Cairns et al (1996) found that RS III was composed of predominantly linear material present in two main molecular size subfractions: one semicrystalline with DP >100 and one with DP 20–30. The results obtained here show there may be a high molecular weight material that can be appreciated in the last part of the profile, because it does not reach the initial baseline. Gidley et al (1995) reported that RS has some periodicity with the local maxima, which is separated by six units; however, such periodicity was not found in this study. DP values obtained for isolated RS from autoclaved and boiled samples did not differ when from the same botanical source and had the same range in all samples.

CONCLUSIONS

The results obtained in this research led us to conclude that RS is highly dependent on botanical origin. No clear relationship was found between RS in native raw material and RS type III yields. Although gelatinization and retrogradation influence RS formation, we conclude that when the same retrogradation procedure was followed in all samples, the variation in method of gelatinization (autoclaving or boiling) did not significantly affect DP for cereal and tuber starch. However, no clear pattern was found in RS yields. Autoclaving led to slightly higher RS levels in wheat and corn, whereas boiling resulted in higher RS in rice. RS yields were not significantly different for potato starch cooked by either method.

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