Resistant Starch Formation: Standardization of a High-Pressure Autoclave Process

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Gelatinization was studied in a high-pressure autoclave (HPA) process. Temperature, stirring, and pressure conditions were continuously controlled during gelatinization. The formation of resistant starch (RS) in standards of potato (amylose, amylopectin, and starch) was investigated during autoclaving in the HPA process and cooling. The results obtained showed that the higher the amylose contents, the larger the RS yield. RS yields obtained in the HPA process were greater than the ones obtained using a boiling water bath as gelatinization system. Scanning electronic microscopy studies of native and retrograded starch showed structural differences between the two states.

Keywords: *Resistant starch; amylose; amylopectin; starch; gelatinization; retrogradation*

INTRODUCTION

Resistant starch (RS) has been recently defined as the sum of starch and its degradation products that are not absorbed in the small intestine of healthy individuals (Euresta, 1992). RS resists digestion and is available for fermentation in the large intestine.

The potential positive effects of RS in health and the possibility of using technological treatments to increase the amount of RS in foods have attracted the attention of nutritionists and food technologists (Annison and Topping, 1994).

RS has been categorized into three main types: I, physically trapped (i.e. partly milled grains and seeds); II, resistant granules (i.e. the native crystalline starch granules in raw potatoes and green bananas); and III, retrograded starch (recrystallized starch after gelatinization and cooling or storage of foods) (Englyst et al., 1992). Additionally chemically modified starch fragments produced by heat treatments, indigestible starch−nutrient complexes, and undigested starch resulting from the action of enzyme inhibitors and antinutrients may contribute to the RS content of a food (Saura-Calixto and Abia, 1991).

Retrograded starch is the most common RS in the diet and, from the technological point of view, it is the most important type because it forms as a result of food processing.

Gelatinization is an essential process that determines the extent of RS formation. When starch granules are fully gelatinized and dispersed, the starch is easily digestible. However, as the gel cools and ages, the polymers again take on a partially crystalline structure (retrograded starch).

The food amylose/amylopectine ratio, along with the temperature and the sample/water volume ratio used in thermal treatments, is closely related to RS yield (Siljeström et al., 1989; Ring et al., 1988; Berry, 1986; Sievert and Pomeranz, 1989).

The gelatinization studies reported in the literature usually treat samples in an autoclave with a constant external temperature, but the internal temperature of the sample and the heating curve are usually not controlled (Berry, 1986; Sievert and Pomeranz, 1989; Eerlingen, 1994). The aim of the present work is to standardize the hydrothermal process in starch gelatinization by using a heating controller high-pressure autoclave (HCHPA) to allow the exact control of temperature, pressure, and stirring within the autoclave.

This technique allows a better technological control of gelatinization and RS formation. The procedure is applied to RS formation from amylose, amylopectin, and potato starch.

MATERIALS AND METHODS

Materials. *Samples.* Potato starch, potato amylose, and potato amylopectin (all from Sigma) were used as standards.

Enzymes. Pancreatic α-amylase (Sigma), pepsin (Merck), amyloglucosidase (Boehringer) were used.

Reagents. An enzymatic kit was used for glucose determination (Peridochrom glucose; GOD-PAP method, Boehringer Mannhein), and D-(+)-glucose (Merck) was the spectrophotometric standard.

All reagents used in this work were of analytic grade.

Apparatus. A high-pressure autoclave (Berthod) was equipped with a pressure glass with vacuum line (PTFE) and thermocouple (DIN 43710), heating cover with magnetic stirring, thermosensor, and temperature control system, and stirring rate control. A second temperature system control was connected to the high-pressure autoclave. The high-pressure autoclave and heat flow are shown in Figure 1.

A UV−visible Lambda 5 (Perkin-Elmer) spectrophotometer was used as was a DSM 950 (ZEISS) scanning electron microscope.

Methods. *RS Formation.* Gelatinization was performed in HCHPA at an initial pressure of 2 bar to avoid glass deformation. To obtain homogeneous gels and complete gelatinization, a stirring speed of 1300 rpm was used. The sample/water ratio was 1:20 (w/v). The gelatinization conditions were $T \geq 115$ °C during 20 min. Immediately, the

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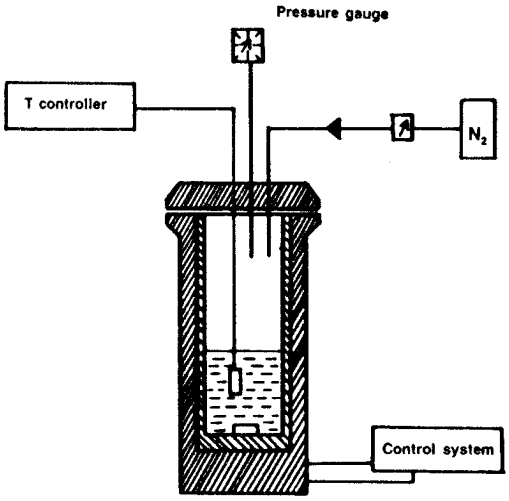

Figure 1. Scheme of high-pressure autoclave.

Table 1. Gelatinization Systems Reported in the Literature

system	temp (°C)	time (min)	ref
boiling water bath	95	30	Ring (1984)
autoclave	121	15	Berry (1986)
autoclave	121, 134, 148	60	Sievert and Pomeranz (1989)
boiling water bath	80[a]	5[a]	
	105[b]	30[b]	l'Anson (1990)
boiling water bath	100	NR[c]	Siljeström (1990)
boiling water bath	100	15	Roulet (1990)
autoclave	121	60	Eerlingen (1994)

[a] First step. [b] Second step. [c] NR; not reported.

samples were cooled to room temperature (cooling rate approximately 4 °C/min) and frozen at −20 °C afterward. After 12 h, the samples were defrosted (defrosting rate approximately 0.4 °C/min) and vacuum-dried at 40 °C for 12 h. Finally, the samples were ground to a particle size ≤1 mm.

RS Determination. RS is considered to be the residue remaining after incubation of the samples with α-amylase at 37 °C for 16 h (pH 6.9). Previously the samples were preincubated with pepsin (40 °C, 1 h, pH 1.5). The residues were dispersed in water before KOH was added to a final concentration of 2 M and incubated with amyloglucosidase for 30 min at 60 °C (pH 4.75). Glucose was measured using an enzymatic kit for glucose/perosidase.

Scanning Electron Microscopy (SEM). Samples were mounted on aluminum specimen stubs with double-sided adhesive tape, sputter-coated with gold using a Polaron E-500, and examined in a ZEISS DMS-950 scanning electron microscope.

RESULTS AND DISCUSSION

Standardization and Gelatinization Conditions. Table 1 summarizes gelatinization conditions published in the literature. As can be observed, there has been no standard procedure. In this paper we propose one.

Different assays were performed using a standardized thermal process and a single recording lector-controller. The mantle was heated at 120 °C and applied for 9−10 min. The temperature was then set at 250 °C. Then the temperature lector-controller was fixed at 50 °C for

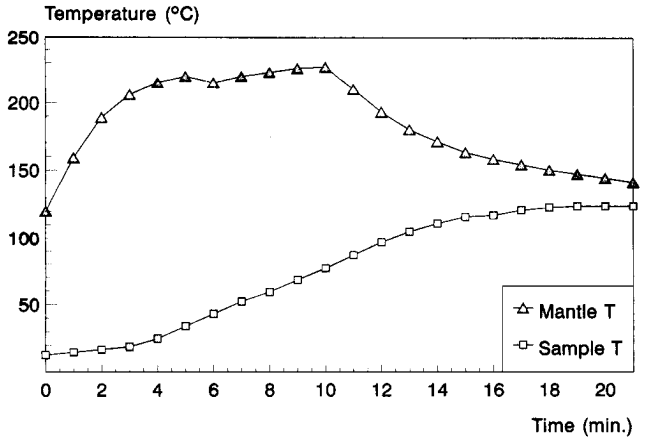

Figure 2. Heating curves by using one temperature lector-controller.

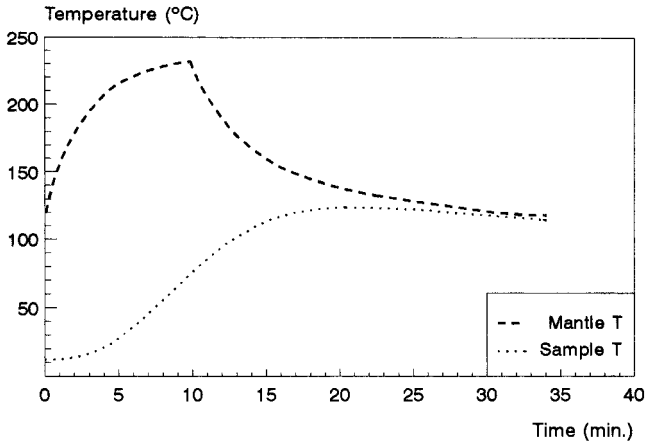

Figure 3. Heating curves by using two temperature lector-controllers.

10 min. The mantle and sample temperature were measured at 1 min intervals. The resulting heating curves are shown in Figure 2. This procedure provides initial precise information on the sample and the heating/cooling thermal curves of the mantle. Nevertheless, the use of just one temperature lector-controller alters heat distribution and makes it difficult to ascertain the precise temperature of the sample. However, these alterations in heat distribution also prevent the mantle from reaching 250 °C, so the sample was heated slowly. These assays did not maintain the sample for 20 min at $T \geq 115$ °C, so a new stabilizing heating step with the temperature lector-controller set at 120 °C was needed. To avoid this, a second temperature lector-controller was connected to the HCHPA.

The use of two lector-controllers allows the simultaneous reading of the sample and the mantle. In these assays after the step at 50 °C, the mantle temperature lector-controller was fixed at 120 °C for 10 min. Figure 3 shows an example of the resulting average temperature curves. As can be observed, the sample reaches a temperature of 80−110 °C after 11 min in the autoclave (amylose, 110 °C; amylopectin, 80 °C; starch, 100 °C). The experiment was maintained at $T \geq 115$ °C for 20 min. Complete gelatinization takes place under these conditions.

Figure 4 shows heating curves obtained with starch, amylose, and amylopectin potato standards. As can be noted, all of the temperature heating curves follow the same tendency and allow one to know the hydrothermal path during the gelatinization process.

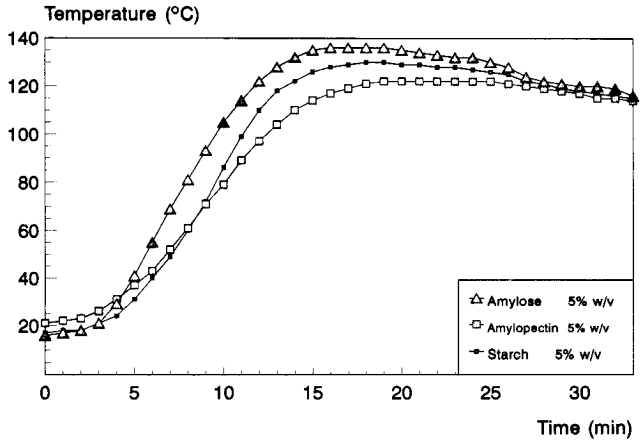

Figure 4. Gelatinization curves in potato standards.

Table 2. RS Yields in Autoclaved and Retrograded Amylose/Amylopectin Mixtures

amylose/ amylopectin (%, dm)	RS[a] (%, dm)	amylose/ amylopectin (%, dm)	RS[a] (%, dm)
100/0	36.45 ± 2.31	40/60	19.07 ± 0.40
75/25	28.06 ± 1.46	25/75[b]	18.16 ± 0.23
50/50	21.48 ± 0.41	15/85	8.97 ± 0.29
		0/100	7.61 ± 0.38

[a] Values are average of three gelatinization treatments in HCHPA. [b] Potato starch.

Table 3. Yields of RS from Sievert and Pomeranz (1989) and Berry (1986)

standard	amylose (%)	RS (%, dm)
A. Sievert and Pomeranz (1989)[a]		
amylomaize VII	70	21.3 ± 0.3
amylomaize V	53	17.8 ± 0.2
pea starch	33	10.5 ± 0.1
wheat starch	25	7.8 ± 0.2
maize starch	26	7.0 ± 0.1
potato starch	20	4.4 ± 0.1
waxy maize	<1	2.5 ± 0.2
B. Berry (1986)[b]		
potato amylopectin	0	2.8
waxy maize	0	0.5
wheat starch	25	4.0
amylomaize V	50	26.2
amylomaize VII	70	28.5
potato amylose	100	31.0

[a] Values are average of three determinations. [b] Values are average of two determinations.

The final pressure was 4 bar, and samples were collected from the HCHPA at $T \leq 100$ °C and $P \leq 3$ bar. Under these conditions a homogeneous gelatinized fraction is obtained and pressure glass deformations are avoided.

RS Formation. RS yields from potato starch and amylose/amylopectin samples that were autoclaved with the HCHPA are shown in Table 2. As can be observed, the higher the amylose contents, the larger the RS yield. These results reveal the important role of amylose in starch gels retrogradation. Berry (1986) and Sievert and Pomeranz (1989) found similar results in starch of different sources (see Table 3). Interestingly, the RS yields shown here are higher than the values reported by other authors using the same samples. These differences are shown in Table 4.

RS formation conditions referred to in the literature are shown in Table 5.

The starch/water ratio used in this work does not explain the differences because RS yields increased with the decrease in the amount of water (Sievert and Pomeranz, 1989). The continuous control autoclaving

Table 4. Differences in RS Yields as Compared to Those of Other Authors

	RS (%)	RS (%)
0% amylose	2.8[a]	7.6[b]
100% amylose	31.0[a]	36.4[b]
potato starch	4.4[c]	18.2[b]

[a] Berry values. [b] Escarpa et al. values. [c] Sievert and Pomeranz values.

Table 5. RS Formation Conditions Reported in the Literature

conditions	Berry (1986)	Pomeranz (1989)	Eerlingen (1994)	Escarpa et al. (1994)
gelatinization (min, °C)	15, 121	60, 134	60, 121	20, 115−136
retrogradation (h, °C)	48, 4	12, 4	48, 25	12, −20
water content (%)	67	75	50	95

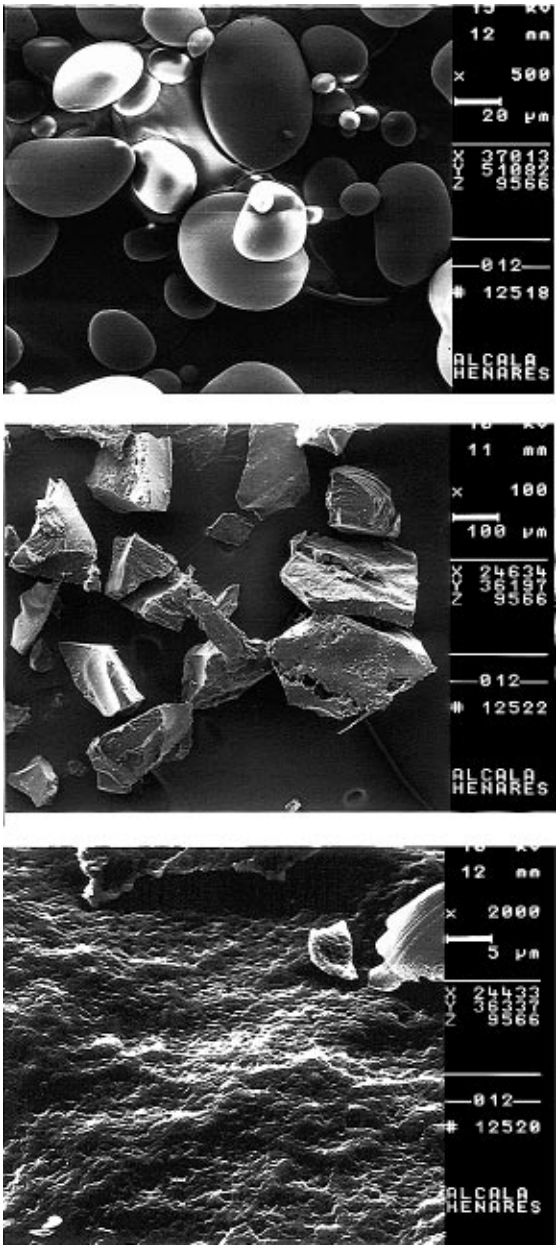

Figure 5. Scanning electron micrograph of native potato starch (top), retrograded potato starch (middle), and retrograded potato starch at higher magnification (bottom).

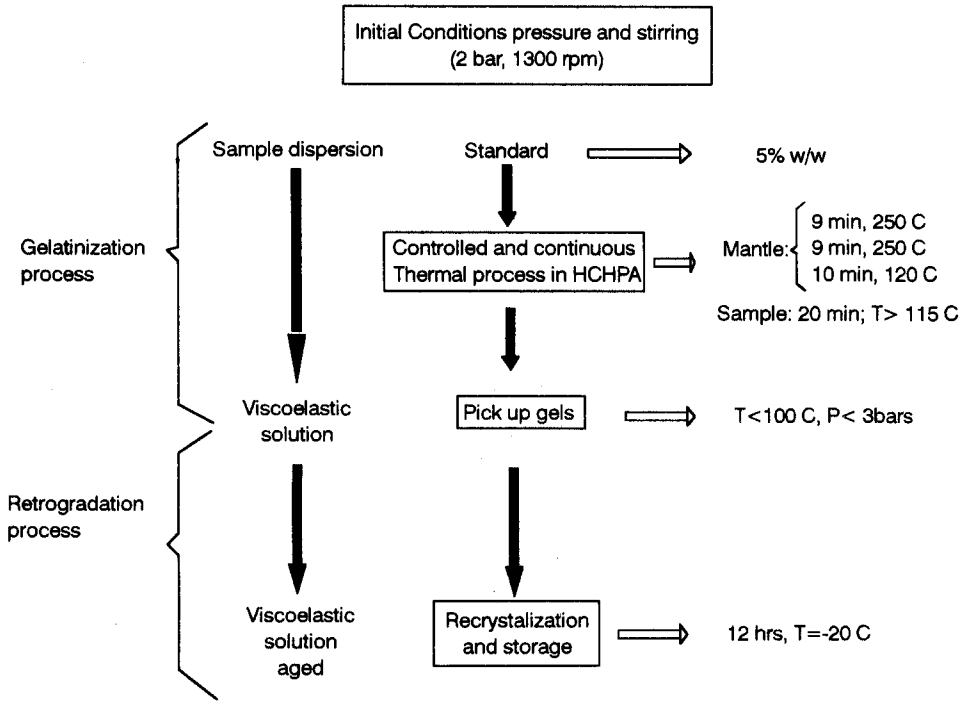

Figure 6. Standardized procedure scheme of RS.

temperature employed in this work (115−136 °C) also accords with previously published temperatures. The differences in RS yields between 121 and 134 °C are small. Increasing autoclaving temperatures (148 °C) decrease the RS yield (Sievert and Pomeranz, 1989).

On the other hand, in this work, a significant role of amylopectin in starch retrogradation was found. In our case, the RS yield of pure amylopectin was 7.61%, which is higher than reported values (see Table 3). Therefore, these results show that amylopectin retrogradation cannot be excluded. This also agrees with recently published studies which reveal that retrograded amylopectin may contain different levels of RS when the storage conditions are optimized to favor amylopectin retrogradation in waxy maize (Eerlingen, 1994).

However, the RS yield obtained in amylopectin retrograded under the storage conditions employed in this work was not expected because the overall crystallization rate mainly depends on the nucleation and propagation rates, which are zero at temperatures below the glass transition temperature (about −5 °C for B-type starch gels) (Slade, 1984).

Therefore, to determine if the differences found in the literature could be due to the gelatinization system, raw potato starch was gelatinized using a boiling water bath as gelatinization system (100 °C, 1 h) under the same RS formation conditions. The yields of RS obtained were 11.60 ± 0.08 ($n = 2$). These values are lower than the ones obtained using HCHPA. This experiment, along with the data found in the literature, indicates that HCHPA makes it possible to obtain higher RS yields than those obtained using a conventional autoclave or boiling water bath as gelatinization system.

SEM Starch Characterization. Images corresponding to raw and retrogaded starch are shown in Figure 5. As can be observed, there are structural differences between the two images. Raw starch has a granular appearance, while in retrograded starch the granular structure disappears. At a higher magnification, irregularly shaped particles with a continuous sponge-like, porous network become visible in the retrograded starch. These results reveal the complete gelatinization of starch in the HCHPA process.

Conclusions. The estimated parameters (pressure and stirring) and the continuous heating conditions used in the HCHPA treatment of starches allow better control of gelatinization and improve RS yields. This procedure can be used for systematic studies of RS formation in foods, which has been summarized in Figure 6.

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