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Assessment of some parameters involved in the gelatinization and retrogration of starch

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Abstract

Factors influencing the formation of resistant starch (RS) during gelatinization and retrogradation were studied in starches and flours from cereals (wheat, corn, rice) and potato. RS obtained using a high-pressure autoclave system varied between 3.94 and 21.21% (rice and potato starches, respectively) similar to the values obtained after gelatinization in a boiling water bath. Except for rice, RS was higher in pure starches than in flours. Stirring during gelatinization yielded more homogeneous products than non-stirred samples. Apparently, gelatinization was unaffected by pH values between 3.5 and 10.5. To obtain optimum RS yields during retrogradation, it was necessary to cool down starch gels prior to freezing, followed by thawing at room temperature and drying at 60°C. These conditions ensure good yields in the formation of RS with potential industrial applications. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

Starch, as the major storage polysaccharide in plants, is contained within granules comprised of amylose and amylopectin chains in ratios relatively constant (about 20:80), depending on the botanical origin. However, mutants with different ratios are known (i.e. waxy starches formed basically by amylopectin or high amylose starches such as amylomaize with about 70% amylose). The form, size and crystalline structure of starch granules also depend largely on their botanical origin. Native starch granules are partially crystalline, with a crystallinity ranging from 15 to 45% (Zobel, 1988). Cereal starches are characterised by an A-type diffractometric spectrum, while tubers have a B-type pattern and legumes exhibit a C-pattern, considered by some authors as a mixture of A and B-type starches (Gallant, Bouchet, Buléon, & Pérez, 1992).

Native starch granules, as appear in raw foods, are mostly indigestible. Gelatinization of starch occurs when foods are heated in an excess of water. During the gelatinization process, starch granules swell and gradually lose their molecular order; the amylose chains solubilize and a starch gel is formed. At this point, starch is easily digestible. Upon cooling, the gel undergoes transformations leading to a partially crystalline structure, both amylose and amylopectin taking part in this process that results in the formation of retrograded starch (Colonna, Leloup, & Buléon, 1992). Retrograded starch, as native starch granules, is also indigestible.

Resistant starch (RS) was defined as the starch and the products of starch degradation that are not absorbed in the small intestine of healthy individuals (Asp, 1992). According to the classification established by Englyst, Kingman, and Cummings (1992), RS can be divided into different types: RS_1 is the physically inaccessible starch found in partly milled grains, RS_2 corresponds with native starch granules and RS_3 is the retrograded starch. Other authors distinguish another type of RS, RS_4 comprised of chemically modified starches (Björck et al., 1987). In processed foods RS is mainly made up of RS_3.

RS reaches the large intestine where it is fermented by the colonic microflora with the production of short chain fatty acids (mainly acetic, propionic and butyric acids), CO_2, H_2 and, in some individuals, CH_4 (McBurney, Cuff, & Thompson, 1990; Cummings & Englyst,

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1991). Several studies have shown that RS may have important repercussions on human health, with some effects similar to those reported for soluble dietary fibre (Stephen, 1991; Muir et al., 1993; Annison & Topping, 1994; Asp, Van Amelsvoort, & Hautvast, 1996). Thus, RS consumption has been related to reduced postprandial glycaemic and insulinemic responses, which may have beneficial implications in the management of diabetes (Granfeldt, Drews, & Björck, 1995). Also, a protective effect against colorectal cancer has been attributed to RS (Roediger, 1982; Cassidy, Bingham, & Cummings, 1994; Hylla et al., 1998), as well as hypocholesterolemic effects (De Deckere, Kloost, & Van Amelsvoort, 1995; Ranhotra, Gelroth, & Leinen, 1997; Vanhoof & De Schrijver, 1997).

Retrograded starch is the most common starch fraction in processed foods and therefore the most important from nutritional and technological points of view. Processing techniques and storage conditions may affect both the gelatinization and retrogradation processes, influencing the RS formation. This fact is of great interest for the food industry, since it offers the possibility of increasing the RS content of processed foods and foodstuffs. Baking, pasta production, extrusion cooking, autoclaving, etc. are known to influence the yield of RS in foods (Siljeström & Asp, 1985; Björck et al., 1987; Siljeström, Eliasson, & Björck, 1989; Muir & O'Dea, 1992; Rabe & Sievert, 1992). On the other hand, a number of factors involved in the gelatinization and retrogradation steps may affect the yield of RS. Several studies report on different levels of RS in autoclaved or boiled wheat samples (Björck et al., 1987; Berry, I'Anson, Miles, Morris, & Russell, 1988; Siljeström et al., 1989). However, slight variations in the conditions of sample treatment (stirring, water/sample ratio, time and temperature of gelatinization, etc.) make difficult the comparison of results. Also other factors, such as the type and concentration of starch, cooking and cooling regimes, pH or the presence of other food constituents (protein, lipids, etc.), can affect the formation of RS (Biliaderis, 1991; Saura-Calixto & Abia, 1991; Eerlingen, 1994; Escarpa, González, Morales, & Saura-Calixto, 1997).

The objective of the present research was to elucidate optimal conditions for the production of RS in a technological process. To this aim, we studied some of the factors supposed to influence the formation of RS during the gelatinization and retrogradation steps.

2. Materials and methods

2.1. Samples

Native wheat, corn, rice and potato starches were purchased from Sigma Chemical Co., St. Louis, MO. Wheat flour (Carret, Torrejón, Madrid, Spain) and corn flour (Santiveri, Barcelona, Spain) were purchased in a local market, while rice (SOS, Algemesí, Valencia, Spain) and potato flours were prepared in the lab. Rice was milled to a particle size of less than 1 mm using a Cyclotec 1093 Sample Mill (Tecator, Höganäs, Sweden). Fresh potato was lyophilised and milled as above to obtain potato flour.

2.2. Sample preparation: formation of resistant starch

2.2.1. Gelatinization

Starches and flours were gelatinised following one of these two methods.

Method 1 used a high-pressure autoclave system (Berghof GmbH, Eningen, Germany), equipped with a pressure glass with vacuum line (PTFE) and a thermocouple (DIN 43710), heating cover with magnetic stirring, thermosensor, and a temperature and stirring control system. Gelatinization conditions were previously standardised (Escarpa, González, Mañas, García-Diz, & Saura-Calixto, 1996). Initial pressure was set at 2 bar with N_2. Stirring speed was 1300 rpm, and gelatinization temperature (120°C) was maintained for 20 min.

In Method 2, gelatinization was carried out in 50 ml centrifuge tubes. Capped tubes containing the starch suspension and a magnetic stirring bar were placed into a boiling water bath for 45 min with constant stirring.

In both methods the sample (5 g) was dispersed in 40 ml of distilled water.

2.2.2. Retrogradation

Gelatinised samples, independently of the gelatinization method used, were poured onto Petri dishes and let to cool at room temperature prior to freezing at −20°C. After 16 h, samples were defrosted at room temperature (8 h) and dried in an air circulating oven (flow rate of 2.3 m^3/min) at 60°C for 16 h. Finally, samples were milled to a particle size $\leq$ 1 mm, using a Cyclotec 1093 Sample Mill.

2.3. Factors affecting resistant starch formation

Several factors that might influence the formation of RS were studied during both gelatinization and retrogradation steps.

(A) Factors involved in starch gelatinization.

- Stirring: gelatinization of native starches was performed with and without a magnetic stirring bar during the autoclave gelatinization step.
- pH: the effect of pH on starch gelatinization was checked using several solutions of different pH (3.5, 5.5, 8.5 and 10.5) instead of distilled water. Acetic acid and KOH solutions were used to obtain acidic and alkaline solutions, respectively.

(B) Factors involved in starch retrogradation. Different variables were tested for their possible influence on starch retrogradation:

- Freezing versus refrigerating: samples frozen overnight at −20°C were compared with other samples kept at 4°C during 14 days. Gelatinised samples were allowed to cool down to room temperature prior to the freezing/refrigerating step, and further dried and milled as described above.
- Cooling/freezing: samples cooled to room temperature and frozen overnight at −20°C prior to drying were compared with (i) samples cooled at room temperature and dried without freezing and (ii) samples dried directly after gelatinization without cooling and freezing.
- Temperature of drying: two drying temperatures were used to dry the retrograded samples: 60 and 100°C.

2.4. Total starch determination

Samples (50 mg) were suspended in 2 M KOH to disperse starch and shaken at room temperature during 30 min. Then, samples were incubated with amyloglucosidase to hydrolyse starch (ref 102857, Boehringer Mannheim, Germany) (60°C, 45 min, pH = 4.75), and glucose was determined using the glucose oxidase assay GOD-PAP (ref 676543, Boehringer Mannheim, Germany). Total starch was calculated as glucose × 0.9. (Goñi, García-Alonso, & Saura-Calixto, 1997).

2.5. Resistant starch determination

Resistant starch (RS) was measured by the procedure of Goñi, García-Diz, Mañas, and Saura-Calixto (1996). In brief, the method has the following steps: removal of protein with pepsin (7190, Merck, Darmstadt, Germany) (40°C, 1 h, pH = 1.5), incubation with α-amylase (Sigma A-3176) at 37°C for 16 h to hydrolyse digestible starch, treatment of the residues with 2 M KOH to solubilize RS, incubation with amyloglucosidase to hydrolyse RS (Boehringer Mannheim, Germany) (60°C, 45 min, pH 4.75), and determination of glucose as described above. RS was calculated as glucose × 0.9.

2.6. Statistics

Results are expressed as mean values ± standard deviation. Comparison of means was performed by one way analysis of variance (ANOVA). STATGRAPHICS Computer System, version 5.1 was used.

3. Results and discussion

Table 1 shows the total starch (TS) content of all the studied pure starches and flours, as well as the resistant starch in the retrograded samples, expressed on a dry weight basis (RS) or as a percentage of the total starch (relative resistant starch, rRS). Samples were gelatinised as described in Method 1, and retrograded.

All the pure starches showed TS values higher than 90.0% dry matter (Table 1). Flours were also very rich in TS (values ranging from 77.8 to 87.5% dry matter). Except for rice, RS formation was more pronounced in starches than in flours. Retrograded wheat and corn starches yielded 14.4 and 11% RS, respectively, whilst rice starch had the smallest amount of RS (3.97%). The highest value corresponded to potato starch (21.2%). As mentioned above, starch granules in potato have a B-type diffractometric pattern, different from the A-type crystalline structure of cereal starches (Gallant et al., 1992). This difference could account for the higher RS yields in potato samples, since starch granules showing the B pattern have been shown to be more resistant to enzymatic hydrolysis than A-type starches (Gallant et al., 1992). Moreover, potato starch granules are bigger than cereal granules (5–100 μm versus 0.5–4.5 μm, respectively), and yet the amylose content is slightly higher in cereal starches; potato amylose chains have a much higher degree of polymerisation (DP) than cereal chains (Sivak & Preiss, 1998). These differences might account for the distinct susceptibility to retrogradation of the starches in cereals and tubers. Differences of the RS formed among the studied cereals and tubers involve variations in the DP, granule structure or properties of the starch gels. More in-depth physico–chemical and structural studies are necessary to clarify these points.

Among the flours, potato also showed the highest RS value. Corn flour had the lowest RS content whilst wheat showed the highest RS formation of the retrograded cereal flours as it did when comparing pure cereal starches. Surprisingly, although rice starch was the sample with the lowest RS yield, retrograded rice flour resulted in a high formation of RS. This is the only

Table 1
Total starch (TS) and resistant starch (RS) content of native and retrograded starches and flours[a]

	TS (% d.m.)	RS (% d.m.)	r RS (% TS)
Wheat			
Starch	90.0 ± 0.56	14.4 ± 0.87	16.0 ± 1.09
Flour	79.5 ± 3.21	7.25 ± 0.56	9.13 ± 0.78
Corn			
Starch	91.3 ± 0.79	11.0 ± 0.80	12.0 ± 0. 96
Flour	86.2 ± 0.43	1.96 ± 0.22	2.27 ± 0. 28
Rice			
Starch	93.0 ± 1.88	3.94 ± 0.50	4.23 ± 0.66
Flour	87.5 ± 1.01	5.44 ± 0.30	6.06 ± 0. 60
Potato			
Starch	91.7 ± 0. 65	21.2 ± 1.30	22.8 ± 1.16
Flour	77.8 ± 0. 85	14.6 ± 0.20	18.7 ± 0.27

[a] Mean values ± STD ($n = 3$).

sample where the RS content of the retrograded flour was higher than in the retrograded pure starch (Table 1). Rice grains are rich in a storage protein called oryzenin (over 80–90% of total proteins) which is known to interact with starch by binding to amylopectin and/or amylose (Chrastil, 1990). This may be responsible for the higher RS formation in rice flour as compared with purified starch where no oryzenin is present.

When RS contents were expressed as values relative to TS, to correct for differences in the starch content of the samples, the same trends were observed (Table 1).

3.1. Factors affecting starch gelatinization

Three factors were studied for their influence on starch gelatinization, in all cases suspending the samples in an excess of water (5 g, 40 ml) and ensuring the temperature of gelatinization (50–70°C in excess of water). The factors studied were: (i) pressure and temperature applied during the gelatinization step, tested using a high pressure autoclave system with an initial pressure at 2 bar and a gelatinization temperature of 120°C and compared with gelatinization at 100°C at atmospheric pressure in a boiling water bath; (ii) stirring (1300 rpm in the autoclave and 1600rpm in the water bath) versus static gelatinization; and (iii) pH, range between 3.5 and 10.5. Results obtained using the two different gelatinization systems with or without stirring are shown in Table 2. No statistically significant differences in the RS formation using either the autoclave system (Method 1) or the boiling water bath (Method 2) were observed. As mentioned before, gelatinization temperature was surpassed in both methods and apparently the pressure applied in the autoclave system did not influence starch gelatinization. Previous results showed no differences in the degree of polymerisation (DP) between autoclaved and boiled samples with an average chain length of 50-60 (García-Alonso, Saura-Calixto, & Delcour, 1998), suggesting that both gelatinization methods led to similar final products. However, the conditions in the autoclave system, previously standardised (Escarpa et al., 1996), are more easily monitored and reproducible. Therefore, we selected this method as the gelatinization procedure to be used for further studies.

Table 2
Comparison of resistant starch yields obtained following method 1 (gelatinization in a high-pressure autoclave system) or method 2 (gelatinization in a boiling water-bath) with and without stirring (% dry matter)[a]

	Gelatinised in autoclave (Method 1)		Gelatinised in water bath (Method 2)
	With stirring	Without stirring	With stirring
Wheat starch	14.4 ± 0.87a	15.9 ± 1.3a	14.7 ± 0.50a
Corn starch	11.0 ± 0.80a	12.1 ± 1.76a	9.76 ± 0.68a
Rice starch	3.94 ± 0.50a	3.87 ± 1.49a	4.41 ± 0.83a
Potato starch	21.2 ± 1.30a	25.1 ± 2.32a	23.6 ± 1.16a

[a] Mean values ± STD ($n = 3$). Different letters in each line show significant differences ($p \leqslant 0.05$).

On the other hand, there were no differences in the RS formed with or without stirring during gelatinization in the autoclave (Table 2). In the case of samples gelatinised in the boiling water bath without stirring, there was very little homogenisation of the starch gel, leading to the separation of two phases, a liquid one and a heterogeneous, highly viscous gel. These samples were not analysed, due to the non-reproducibility of the resulting product. Stirring ensures an adequate homogenisation of the starch/water suspension and thus the formation of a more homogeneous gel. It also improves heat transference between different layers of the starch gel. Studies reported in the literature do not clearly state whether or not samples were stirred during all the gelatinization process (Björck et al., 1987; Berry et al., 1988; Sievert & Pomeranz, 1989; Siljeström et al., 1989). Only in some cases initial stirring of the starch suspension is mentioned (Miles, Morris, Orford, & Ring, 1985; Cooke & Gidley, 1992). Results from this study (Table 2) suggest that the sample should be stirred during gelatinization in order to achieve homogeneous gels and more standardised products.

Samples used to study the influence on gelatinization of the pH of the solutions used to disperse starch were gelatinised in the water bath instead of the autoclave because this saved time and allowed higher number of samples to be processed at a time. Gelatinization using the autoclave was only 20 min against 45 min in the water bath. However, before opening the autoclave it was necessary to allow some time for it to cool down in order to prevent sample loss due to overpressure within the autoclave pressure glass, which lengthened the total time needed for the treatment. Moreover, the autoclave only allowed gelatinization of one sample at a time, whilst several samples could be gelatinised in parallel in the boiling water-bath. Only corn starch showed statistically significant differences in the RS formation at different pH values (Table 3). When using solutions of pH 3.5, 5.5 and 8.5, higher RS yields were obtained than when solutions of pH 7 and 10.5 were used. Nevertheless, this increase, which was not quantitatively important, occurred in one particular sample and no clear relationship between acid or basic pH and this change in the RS content could be established. Therefore, it may be assumed that, in general, this pH range does not affect starch gelatinization and subsequent RS formation. This fact is important from an industrial point of view, because it shows that good RS yields can be obtained by suspending starch directly in water with the subsequent savings and facility of the technological process. However, pH values below 1.5 and above 13

may cause hydrolysis and solubilisation, respectively, of the sample (data not shown).

3.2. Factors affecting starch retrogradation

Retrogradation is a complex process in which amylose chains, solubilized during gelatinization, aggregate, forming crystalline double helices stabilised by hydrogen bonds (Jane & Robyt, 1984). Upon cooling and ageing, these helices aggregate to form three-dimensional crystalline structures of the B-type (Miles et al, 1985). These crystallites are highly stable, showing a melting endotherm at about 150°C, and are resistant to enzyme digestion. Amylopectin molecules can also crystallise by association of the short lateral chains (Eerlingen, 1994). Whilst amylose retrogradation is a rather fast process taking place in few hours, amylopectin requires longer times (days or weeks). Amylopectin crystallites are less stable than amylose ones, with a melting point close to 60°C. Therefore, storage conditions (time and also temperature of storage) are important factors in the retrogradation process.

To obtain retrograded starch or flour powders suitable for use as food ingredients, retrograded gels need to be dehydrated prior to milling. Two different drying temperatures have been studied, 60 and 100°C. When samples were dried at 100°C there was a general reduction in the RS formation; this decrease was statistically significant in all cases except in corn starch and flour and wheat flour (Table 4). High drying temperatures can result in the melting of retrograded amylopectin crystals that are unstable, as mentioned before (Biliaderis, 1991; Eerlingen, 1994). Therefore, drying temperature should be kept low to avoid RS decreases, which also results in economic savings derived from lower energy consumption during drying.

Drying conditions described in the literature vary from freeze-drying (Siljeström et al., 1989; Sievert & Pomeranz, 1989; Cooke & Gidley, 1992), drying in a vacuum oven (Escarpa et al., 1996) or oven-drying at different temperatures (Berry, 1986; Siljeström et al., 1989). Both freeze-drying and vacuum-drying are time-consuming methods that require expensive equipment and thus are not feasible from a technological point of view, unless the amount of RS formed justifies their use. In a previous work, drying in a vacuum oven at 40°C, it was found that the yield of RS using potato starch was slightly lower than when samples were dried at 60°C (Escarpa et al., 1996). We have also assayed lyophilisation as a drying procedure with two samples, but the yield of RS was much lower than when samples were heated at 60°C (7.2 and 0.92% in wheat and rice starches, respectively). Retrogradation implies the formation of crystals initiated in what is called a nucleation

Table 3
Effect of the pH of the water/starch suspension on the formation of resistant starch (% dry matter)[a]

	pH				
	3.5	5.5	7.00	8.5	10.5
Wheat starch	14.3 ± 0.63b	14.5 ± 1.52b	14.7 ± 0.50b	13.1 ± 2.10b	12.1 ± 1.02b
Corn starch	12.9 ± 0.02c	12.5 ± 1.74c	9.76 ± 0.68b	13.9 ± 2.10c	10.3 ± 0.40b
Rice starch	4.29 ± 0.71b	3.80 ± 0.05b	4.41 ± 0.83b	4.46 ± 0.27b	4.53 ± 0.18b
Potato starch	22.2 ± 1.21b	23.3 ± 1.84b	23.6 ± 1.16b	24.0 ± 0.26b	24.4 ± 0.55b

[a] Mean values ± STD ($n = 3$). Different letters in each line for each factor show significant difference ($p \leqslant 0.05$).

Table 4
Influence of storing conditions and drying temperature on the formation of resistant starch in starches and flours (% dry matter)[a]

	Retrogradation conditions[b]				
	C/F/D_{60}	C/F/D_{100}	C/R/D_{60}	C/nF/D_{60}	nC/nF/D_{60}
Wheat starch	14.4 ± 0.87c	11.0 ± 0.32d	13.2 ± 0.25e	11.0 ± 0.55d	9.95 ± 0.60f
Wheat flour	7.25 ± 0.56c	7.10 ± 0.66c	ND	6.49 ± 0.92d	8.21 ± 0.72e
Corn starch	11.0 ± 0.80c	10.7 ± 1.00d	9.37 ± 0.47d	10.7 ± 0.70c	12.8 ± 0.84e
Corn flour	1.96 ± 0.22c	2.2 ± 0.32c	ND	1.80 ± 0.46c	2.31 ± 0.63c
Rice starch	3.94 ± 0.50c	2.61 ± 0.24d	3.85 ± 0.26c	2.51 ± 0.26d	1.51 ± 0.04e
Rice flour	5.44 ± 0.33c	2.50 ± 0.34d	ND	3.34 ± 0.12e	3.61 ± 0.51e
Potato starch	21.2 ± 1.30c	13.0 ± 0.62d	27.1 ± 0.42e	18.9 ± 0.67f	16.3 ± 0.23g
Potato flour	14.6 ± 0.20c	10.8 ± 0.74d	ND	12.0 ± 1.14e	10.2 ± 0.50d

[a] Mean values ± STD ($n = 3$). Different letters in each line for each factor show significant difference ($p \leqslant 0.05$).

[b] C: cooling (room temperature); nC: not cooling; F: freezing (−20°C, 16 h); nF: not freezing; R: refrigerating (4°C, 14 days); D_{60}: drying at 60°C; D_{100} drying at 100°C. ND: not determined.

process. This is followed by a propagation step during which growth of crystals from the nuclei takes place. In this step, temperatures over 60°C enhance the crystallisation process, whilst at low temperatures (0°C) the propagation of amylose crystals is limited (Eerlingen, 1994). This could account for the low RS yields during lyophilisation due to the low temperatures used in this process.

In another experiment instead of freezing at −20°C during 16 h (CDF_{60}), gelatinised samples were refrigerated at 4°C for 14 days (CRD_{60}). In both cases, samples were allowed to cool to room temperature after gelatinization and prior to freezing/refrigerating and dried afterwards at 60°C. Results are shown in Table 4. Refrigeration appeared to enhance RS formation in potato starch, whilst this was reduced in all the cereal starches, although this reduction was very small and not statistically significant in rice starch. These contradictory results might be due to differences in the botanical origin as well as to variations in the amylose and amylopectin ratios, DP or the structure of the starch granule, among other factors.

To study further the effect of storage conditions on starch gels' retrogradation, samples were dried immediately after gelatinization without previous cooling and freezing (Table 4) or after cooling down starch gels to room temperature before drying but without freezing. The resulting values obtained were compared with those obtained when samples were cooled and frozen before drying. In all cases samples were dried at 60°C. When the comparison was established between samples cooled to room temperature and frozen (CFD_{60}) and those samples cooled but not frozen ($CnFD_{60}$), the yields of RS were significantly higher in the first case, except for corn starch and flour, in which the reduction of RS formed without freezing was not statistically significant (Table 4). The analysis of the results obtained with samples that did not follow any of the processes (cooling and freezing) and were dried right after gelatinization ($nCnFD_{60}$) also showed a tendency to lower RS yields more than those obtained from the samples that underwent any of the treatments explained above. Only in wheat flour and corn starch were the RS values higher than in samples cooled with or without freezing. Corn flour also showed a higher, yet not significant, content of RS, when dried immediately after gelatinization than when cooled and frozen (Table 4). Björck et al. (1987) found that wheat starch samples frozen immediately after autoclaving yielded lower RS levels than those cooled to room temperature and frozen afterwards, which is in agreement with our results. This redounds in the fact that storing time is needed to achieve retrogradation of amylose and amylopectin. Our results suggest that a cooling and freezing step should be included for optimal RS formation during starch retrogradation.

4. Conclusions

In the present work, the influence of different factors on the essential steps (gelatinization and retrogradation) leading to the formation of RS has been studied. The results obtained from different experiments allow us to suggest a basic procedure that could be applied to obtain a high yield of RS aiming at its use as a food ingredient. This procedure consists of gelatinization of a starch suspension in excess of water with constant stirring to obtain a homogeneous gel. The gelatinised sample should be allowed to cool at room temperature before overnight freezing at −20°C to help retrogradation of the starch gel. Drying retrograded starches should be carried out at low temperatures to avoid losses of retrograded sample. A drying temperature of 60°C would be the most appropriate way of dehydrating the sample prior to milling.

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