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Resistant starch formation does not parallel syneresis tendency in different starch gels

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Abstract

The retrograded resistant starch contents (RS-III) and syneresis indices of cold stored starch gels were compared. Isolated starches from three cereals (maize, sorghum and rice), two legumes (jack bean and lentil) and arracacha roots (*Arracacia xanthorrhiza*) were hydrated and gelatinized by boiling. Drained gels were stored for 24 h at 4 °C before the analyses. Neither apparent amylose contents nor water exclusion values showed clear correlation with RS-III content in the overnight stored gels. Legume starches reached 6–7% (dmb) RS-III levels, while the lowest values (2–3.6%) were recorded for maize, rice and arracacha samples. Jack bean starch gels showed the greatest syneresis indices, followed by the cereals, arracacha and lentil preparations. Data support the perceived idea of different mechanisms governing syneresis and RS-III formation in gelatinized starches.

Keywords: Starch gels; Resistant starch; Syneresis; Retrogradation; Gel ageing

1. Introduction

Although starch has been extensively investigated from the chemical and technological points of view, its detailed nutritional characteristics have been, until recently, largely ignored. The discovery of variable digestion rates for apparently similar starches, and the limited *in vivo* digestibility of the biopolymer, have led to important changes in our perception of the physiologically relevant features of dietary starches (Björck & Asp, 1994; Englyst, Kingman, & Cummings, 1992). For example, the existence of indigestible—or resistant—starch fractions, and the influence of food processing and storage conditions on their relative abundance, have been demonstrated (Asp & Björck, 1992; Champ, Martin, Noah, & Gratas, 1999; Vasanthan & Bhatty, 1998).

Gelatinization converts starch into a physical form that is desirable in many food systems. Starch gels are, however, thermodynamically unstable and undergo changes affecting their technological suitability (Lapasin & Pricl, 1995). Upon cooling, starch molecules reassociate in a complex recrystallization process known as retrogradation, which is often associated with water separation from the gel (syneresis; Hoover & Manuel, 1995; Whistler & Daniel, 1985; Yeh & Yeh, 1993). These changes may result in textural and visual gel deterioration (Fredriksson et al., 2000; Thomas & Atwell, 1999). Retrogradation is also important from a nutritional point of view, since most of the resistant starch occurring in processed foods consists of retrograded α-glucans (Champ et al., 1999; García-Alonso, Jiménez-Escrig, Martín-Carrón, Bravo, & Saura-Calixto, 1999). Despite the attention paid to factors affecting retrograded indigestible starch levels in processed foods and starches (Björck & Asp, 1994; Escarpa, González, Morales, & Saura-Calixto, 1997; Fredriksson et al., 2000; García-Alonso et al., 1999), possible relationships between resistant starch formation and other phenomena associated with retrogradation, such as syneresis, have not been investigated.

Starch preparations, showing distinctive and stable physicochemical features, are currently obtained through different ways of modification, but the modified products are often costly and must be subject to food safety regulations (Thomas & Atwell, 1999). Thus, the

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search for substitute industry-suitable native starches is compelling and favours the characterization of novel starch sources, such as the roots and seeds of many wild and yet underexploited crops (Carioca, Arora, Pannir-Selvam, Tavares, Kennedy, & Knill, 1996; Pérez, Breene, & Bahnassey, 1998).

Within this framework, the objective of this work was to determine whether the tendencies of various starch gels to generate resistant starch were related to their proneness to undergo syneresis upon cold storage, changes that have important technological and nutritional implications. Two commercially available and four less studied starch sources were investigated.

2. Materials and methods

2.1. Starch sources

Jack bean seeds (*Canavalia ensiformis* cv. Yaracuy) were supplied by the Faculty of Agronomy, Universidad Central de Venezuela (Maracay). Arracacha—also known as Peruvian carrot—roots (*Arracacia xanthorrhiza*) and lentils (*Lens culinaris*) were purchased from the local market in Caracas, whereas sorghum (*Sorghum* sp.) grains were commercially grown at El Tigre (Anzoategui State, Venezuela). Maize (*Zea mays*) and rice (*Oryza sativa*) were commercial starch preparations supplied by Alfonzo Rivas C.A. (Turmero) and MANPA C.A. (Caracas), respectively.

2.2. Isolation of starch

Jack bean, lentil, arracacha and sorghum starches were isolated following the general procedure described by Pérez, Bahnassey, and Breene (1993). Arracacha starch has been partially characterized in this laboratory (Pérez et al., 1998; Pérez, Borneo, Melito, & Tovar, 1999).

2.3. Starch gel preparation

Eight percent (w/v) suspensions of each starch were prepared by full dispersion of the sample in 0.1 M phosphate buffer pH 6.9, using 30 ml glass centrifuge tubes and applying vigorous magnetic stirring. Samples were incubated for 30 min in a boiling water bath stirring every 10 min, cooled down to room temperature and centrifuged (1500×***g***, 30 min, 4 °C). Unabsorbed water (supernatant) was discarded (Yeh & Yeh, 1993).

2.4. Syneresis

Freshly centrifuged gels were weighed and stored at 4 °C. After 24 h, the amount of water exuded from the gel was measured as described by Yeh and Yeh (1993): excluded water was obtained by centrifugation (1500 ×***g***, 30 min, 4 °C) and its weight was used to calculate syneresis degree, expressed as a percentage of the original gel mass. The drained gel was assessed for resistant starch, as described later.

2.5. Starch, amylose and fat content

Total starch content was estimated in 2 N KOH pretreated samples (Tovar, Björck, & Asp, 1990) by the enzymatic method of Holm, Björck, Drews, and Asp (1986). Retrograded resistant starch-also called type III resistant starch (Englyst et al., 1992) was assessed in dietary fibre residues according to Saura-Calixto, Goñi, Bravo, and Mañas (1993). Apparent amylose content was estimated colorimetrically after iodine binding (Juliano, 1971), using potato amylose (Sigma Chemical Co., St. Louis) as standard. Total fat content was measured according to Schoch (1964).

2.6. Statistical analysis

Means were compared by one-way analysis of variance, followed by the Duncan multiple comparison test, using the Number Cruncher Statistical System (NCSS 5.1).

3. Results

The starch content of the isolated preparations is shown in Table 1. Sorghum and jack bean starches showed the lowest α-glucan levels, which were slightly above 90% dry weight basis. No significant differences in total fat were recorded among the various samples, exhibiting typical low values in the 0.06–0.09% (dmb) range. Thus, lipid interference with the different analyses performed can be ruled out.

In contrast, ample variation was observed in the apparent amylose content of the preparations (Table 1), with the leguminous materials (lentil and jack bean

Table 1
Enzymatically available starch and apparent amylose content of isolated starches

Starch source	Starch[a] (g/100 g dry sample)	Amylose[b] (g/100 g starch)
Corn	97.0 (2.0)c	37.1
Rice	97.1 (1.2)c	34.1
Sorghum	90.7 (0.7)d	17.1
Arracacha	95.8 (0.6)c	21.2
Lentil	95.7 (0.6)c	47.5
Jack bean	90.4 (1.5)d	40.3

[a] Values are means of at least four determinations ± S.D. Means not sharing the same letter are significantly different ($P < 0.05$).
[b] Values are means of two determinations.

Table 2
Retrograded resistant starch (RS-III) content and syneresis indices of 24 h stored gels

Starch gel	RS-3[a]	Syneresis[b]
Corn	2.2	2.1
Rice	3.6	4.6
Sorghum	4.1	1.4
Arracacha	3.3	1.2
Lentil	6.6	1.0
Jack bean	7.0	7.1

[a] g/100 g, dry matter basis. Values are means of at least two determinations; maximal deviation accepted was 0.2%.

[b] g separated water/100 g gel. Values are means of three determinations.

starches) showing the highest values (over 40%). Maize and rice starches had intermediate contents, whereas arracacha and sorghum samples showed the lowest levels (21 and 17%, respectively).

The retrograded resistant starch (RS-III) content of starch gels kept at 4 °C for 24 h varied markedly (Table 2). Legume starches reached 6–7% (dmb) levels, while the lowest values (2–3.6%) were recorded for maize, rice and arracacha samples. Sorghum starch gel exhibited an intermediate RS-III content.

The syneresis index, after 24 h-storage, was high for jack bean starch gels, followed by the cereal samples, whereas arracacha and lentil preparations exhibited the smallest amount of exuded water (Table 2).

4. Discussion

Current trends in the food industry are increasingly influenced by nutritional concerns in addition to technological criteria. Hence, modern concepts of the physiological role of carbohydrates have not passed unnoticed by food producers (Björck & Asp, 1994). This is particularly relevant in the case of unavailable carbohydrates, where dietary fibre and resistant starch attract great attention and research efforts (Saura-Calixto, García-Alonso, Goñi, & Bravo, 2000; Thomas & Atwell, 1999; Tovar, 1994). The present report compared six different starches regarding their gel stability and tendency to generate retrograded—or type III—resistant starch (RS-III) upon storage.

All the isolated starch preparations showed total starch contents above 90% dry matter (Table 1). According to recent studies (García-Alonso et al., 1999; Vasanthan & Batthy, 1998), these purity levels may be considered satisfactory.

The existence of various resistant starch forms in foods is currently recognized (Björck & Asp, 1994; Englyst et al., 1992; Saura-Calixto et al., 2000). However, wet heat-treated isolated starch preparations are likely to contain essentially RS-III, i.e. firmly retrograded starch fractions (Champ et al., 1999; Vasanthan & Batthy, 1998), which corresponds to the resistant starch measured by the analytical method employed here (Champ et al., 1999; Saura-Calixto et al., 1993; Tovar, 1994). Present data show that gels of legume starches, i.e. those from jack beans and lentils, developed significantly higher RS-III levels (Table 2), which is in accordance with the well known tendency of leguminous materials to produce retrograded indigestible starch (Bravo, Siddhuraju, & Saura-Calixto, 1998; Tovar & Melito, 1996; Velazco, Rascón, & Tovar, 1997).

Syneresis is a consequence of the continuous reassociation and eventual recrystallization—or retrogradation—of gelatinized starch polymers during cooling and storage, a process that excludes water from the gel phase (Hoover & Manuel, 1995; Lapasin & Pricl, 1995, Thomas & Atwell, 1999; Whistler & Daniel, 1985). Since resistant starch formation also relates to retrogradation (Englyst et al., 1992; Champ et al., 1999; Thomas & Atwell, 1999), the proclivity of a certain starch gel to exclude water might be expected to rise with its facility to form RS-III. However, the comparison of these parameters for the six overnight-stored starch gels (Table 2) does not support this idea. Jack bean starch, for instance, showed the highest syneresis and RS-III values of all six samples, while lentil starch was close to jack bean in RS-III formation but excluded the smallest amount of water. Maize starch, on the other hand, produced notably less RS than any other sample, despite its medium syneresis value. Evidently, water exclusion from a starch gel system cannot be used to forecast its RS-III forming ability.

These results might be related to the different retrogradation characteristics of the two starch components. Amylose retrogrades quickly and is responsible for textural changes occurring in starch gels during the first few hours of cold storage, whereas the aggregation of amylopectin may have an influence on the paste physical properties over longer periods (Jacobson, Obanni, & BeMiller, 1997; Kalichevsky, Orford, & Ring, 1990; Morris, 1990). Thus, RS-III levels may reflect the rapid and firm recrystallization of amylose (Björck & Asp, 1994; García-Alonso et al., 1999; Vasanthan & Bhatty, 1998), while the syneresis, recorded after 24-h storage, could be essentially due to amylopectin retrogradation, whose controversial and apparently marginal role in resistant starch formation has been discussed recently (Fredriksson et al., 2000).

When the propensity of the six starches to form RS-III was compared with their apparent amylose contents, no evident trend could be obtained. Moreover, the maize starch gel had the smallest RS-III level value after 24-h storage (Table 2) despite an amylose index that was markedly above those recorded for rice, arracacha and sorghum samples (Table 1).

The poor correlation between amylose content and RS-III formation pattern does not agree with previous observations indicating that high amylose starches show greater proclivities to generate resistant starch than corresponding 'low' or 'normal' amylose varieties (Björck, Eliasson, Drews, Gudmunsson, & Karlsson, 1990; Escarpa, González, Mañas, García-Diz, & Saura-Calixto, 1996; Sambucetti & Zuleta, 1996). This apparent contradiction, however, might be related to the different comparisons made in every case, as each of the above referred studies looked at different varieties/genotypes of a single biological species, whereas the present investigation deals with starches isolated from members of various plant families. Vasanthan and Bhatty (1998), found no correlation between the amylose content of barley, maize, field pea and lentil starches and their ability to produce RS-III upon gelatinization/storage, a fact that was explained by putative differences in granular swelling, amylose chain length and amylose leaching patterns among starches from different species. Similarly, the amount of indigestible starch formed after prolonged freezing of cooked cereal and legume-based Brazilian foods did not increase with the amylose content of their starches (Mataruco-Rosin, 2000). Apparently, factors other than the amylose:amylopectin ratio may affect the formation of RS-III in starches from different botanical origins, a subject that deserves more detailed investigation.

Present observations are in accordance with perceived mechanistic differences between the syneresis and RS-III formation processes. Further studies on factors that affect retrogradation, such as pH and concentration of the starch gel, molecular features of its polymer components, the gelatinization procedure employed, presence of non-starch constituents and storage conditions (Escarpa et al., 1997; Fredriksson et al., 2000; García-Alonso et al., 1999), are needed for a better understanding and control of RS-III generation.

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