

In vitro digestibility of edible films from various starch sources

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Abstract

Banana, maize, potato and sagu starches were boiled in the presence or absence of plasticizer (glycerol), producing edible films. In vitro digestibility features, amylose content and amylopectin gel filtration behavior of films and parent starches were evaluated. Available starch contents were lower in glycerol-containing films, due to dilution by the plasticizer. Total resistant starch increased in the maize starch-based film but decreased markedly in those prepared from the other starches. Amylose content of banana starch (~40%) was about double those of the other starches. Nonetheless, all starch films exhibited similar retrograded resistant starch content. Although film production led to increased α -amylolysis rates, these were further augmented by additional film heating, thereby indicating that film-manufacture did not promote complete starch gelatinization. Gel filtration chromatography suggested amylopectin depolymerization after film-making, which may also increase digestion kinetics. The presence of glycerol in the films slowed down starch digestion, a feature of potential dietetic use.

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1. Introduction

The use of edible films and coatings in the food industry is increasing (Tharanathan, 2003). These films are prepared from a variety of components, such as polysaccharides, proteins and lipids, and have potential to extend the food shelf life (Arvanitoyannis & Biliaderis, 1998; García, Martino, & Zaritzky, 2000).

Among polysaccharides, starch is commonly used for the elaboration of edible films, which are characterized by their hydrophilic behavior and their ability to act as oxygen barriers. However, in order to attain control of the mechanical properties of starch films, the addition of plasticizers, such as glycerol, is often necessary (Forsell, Lahtinen, Lahelin, & Myllärinen, 2002). The synergistic action of water and glycerol, as well as other polyols, plasticizes starch (Psomiadou, Arvanitoyannis, & Yamamoto,

1996). These agents decrease intermolecular attractions between the polymer chains, which reduces internal hydrogen binding and produces highly mobile regions due to moisture uptake. As a consequence, film flexibility increases and the tensile strength is reduced (Arvanitoyannis, 1999; Arvanitoyannis & Biliaderis, 1999; Donhowe & Fennema, 1993; Gontard, Guilbert, & Cuq, 1993; Psomiadou et al., 1996).

The addition of chemical agents to starch can modify its susceptibility to enzyme digestion (Tovar, Melito, Herrera, Laurentín, & Pérez, 1999). For instance, Han and BeMiller (2007) combined cross-linking and chemical stabilization of purified starches in order to modulate both the rate and the extent of in vitro amylolysis. Similarly, the addition of plasticizers may modify not only the film flexibility but also its nutritional, functional and organoleptic properties (Donhowe & Fennema, 1993). Changes in the nutritional properties of starch-containing products relate to altered bioavailability of the polymer (Björck & Asp, 1994), a possibility that has not been evaluated for starch-based edible films.

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Until a couple of decades ago, starch was considered to be completely digestible and absorbable in the human small intestine (Björck, Granfeldt, Liljeberg, Tovar, & Asp, 1994). However, more recent work has shown that the digestibility of this polysaccharide varies widely. Structural, compositional and fine chemical characteristics of the starch polymers and certain properties of the starch-containing food are among the reasons for this variable digestibility (Björck et al., 1994; Tovar, 2001). In fact, food form, starch supramolecular arrangement, degree of crystallinity and retrogradation have been identified as major determinants of the extent of starch digestion and absorption in the small intestine. Recently, Liu, Gu, Donner, Tetlow, and Emes (2007) reported that the amylose content and branch chain length of amylopectin may also influence the digestibility features of wheat starch. Hence, from the nutritional point of view, starch has been classified in three groups: rapidly digestible, slowly digestible and indigestible or resistant starch (RS) (Englyst, Kingman, & Cummings, 1992). Resistant starch is defined as the sum of starch plus starch degradation products not absorbed in the small intestine of healthy individuals (Asp, 1992). The main classification of RS was proposed by Englyst et al. (1992); it is based on the nature of the starch and its environment in the food. RS1 corresponds to physically inaccessible starches entrapped in a cellular matrix, as in legume seeds (Tovar, Björck, & Asp, 1992). RS2 are native, uncooked granules of starch, such as raw potato or banana starches, whose crystallinity makes them poorly susceptible to hydrolysis (Englyst & Cummings, 1987; Faisant et al., 1995). RS3 are retrograded starches, which may be formed in cooked foods that are kept at low or room temperature (Noah et al., 1998).

RS can be fermented to a variable extent by the colonic microflora, yielding short fatty acids, with important implications for human health (Björck & Asp, 1994; García-Alonso & Goñi, 2000).

A tool for ranking foods with respect to their blood glucose raising potential is the glycemic index (GI) (Jenkins et al., 1981). RS content is a nutritional variable that may be linked to low GI foods. Hence, the RS could be considered as a possible way for improving the dietary control of diabetics (Björck & Asp, 1994; Björck et al., 1994).

It is important to mention that the distribution of the nutritionally relevant fractions of starch in foods is significantly affected by processing (Björck et al., 1994; Carmona-García, Osorio-Díaz, Agama-Acevedo, Tovar, & Bello-Pérez, 2007; Englyst et al., 1992). Thus, depending on the processing protocol and subsequent handling, marked bioavailability differences may be observed among products prepared from similar ingredients. Research on the digestibility of edible films is scarce. Ou, Kwok, and Kang (2004), for instance, evaluated in vitro digestibility changes of soy protein isolate when incorporated into a film, concluding that protein digestibility decreased after film formation.

The objective of the present study was to evaluate the influence of the production of films on the in vitro digestibility of starches isolated from four botanical sources. Attention was paid to the effect of incorporating glycerol as the film plasticizer.

2. Materials and methods

2.1. Materials

Sagu (*Canna edulis*) and potato (*Solanum tuberosum*) were purchased from the local market in Caracas. Decorticated rhizomes and tubers, respectively, were used to isolate starch, as described by Pérez, Bahnassey, and Breene (1993). Green cooking bananas – or “plantain” – (*Musa paradisiaca*) were also from commercial origin and their starch was isolated according to Bello-Pérez, Agama-Acevedo, Sánchez-Hernández, and Paredes-López (1999). Commercial maize (*Zea mays*) starch was supplied by Alfonzo-Rivas & Co., Caracas, Venezuela.

2.2. Methods

2.2.1. Amylose content

Apparent amylose content was estimated colorimetrically after iodine binding (Juliano, 1971), using potato amylose (Sigma Chemical Co., St. Louis) as a standard. Additionally, gel filtration chromatography (see Section 2.2.4) was also employed for evaluating the amylose/amylopectin ratio. In the last method, fractions corresponding to the main peak ($-0.03 \geq K_{av} \geq 0.03$) were considered to be amylopectin, whereas those eluting with $K_{av} > 0.03$ corresponded to amylose. The amylose/amylopectin ratio was calculated from the sum of total carbohydrates eluted under each peak.

2.2.2. Film preparation

Three grams of starch were mixed with distilled water (100 mL) and 1.5 mL glycerol at room temperature (25 °C) for 5 min. This suspension was transferred to a water bath at 98 °C for 30 min, with frequent magnetic agitation (500 rpm). Five milliliters of the gelatinized suspension were immediately poured onto a Petri dish (5 cm in diameter) and placed in a refrigerator until dry (10 days) at 4 °C. The samples were further dried at 40 °C for 48 h and placed in a desiccator with activated silica gel. Samples were milled and stored in a desiccator at room temperature for further analysis.

2.2.3. In vitro digestibility tests

Potentially-available starch content was assessed following the multienzymatic protocol of Holm, Björck, Drews, and Asp (1986) using Termamyl® (Novo A/S, Copenhagen) and amyloglucosidase (A-7255: Sigma Chemical Co., USA). Resistant starch was assessed by two different protocols: (1) retrograded resistant starch (RS3) content was measured as starch remnants in dietary fiber residues,

according to the so-called “Lund method”, as modified by Saura-Calixto, Goñi, Bravo, and Mañas (1993); (2) the method proposed by Goñi, García-Diz, Mañas, and Saura-Calixto (1996) was employed to estimate total indigestible starch content (comprising part of the RS1 plus RS2 and RS3 fractions; (Tovar, 2001)). The in vitro rate of hydrolysis was measured using hog pancreatic amylase according to Holm, Björck, Asp, Sjöberg, and Lundquist (1985); each assay was run with 500 mg available starch.

Before each test, samples were suspended in the appropriate solution and homogenized with a Tekmar tissue homogenizer (Germany), applying three 1-min pulses at speed level 5 (Tovar, Björck, & Asp, 1990).

2.2.4. Gel filtration chromatography

Gel filtration chromatography was performed using a 1.6×80 cm Sepharose CL-4B column, equilibrated with 0.1 M KOH. Each assay was run with 70 mg starch dispersed in 2 M KOH, and eluted at 0.25 mL/min collecting 2 mL fractions (Schweizer & Reimann, 1986). Total carbohydrate in each fraction was assessed according to the anthrone-sulfuric acid method (Roe, 1955). In order to allow for the calculation of K_{av} , the column was calibrated with blue dextran (Sigma Chemical Co., St. Louis) for V_0 , and glucose (Sigma Chemical Co., St. Louis, MO) for $V_0 + V_i$.

2.2.5. Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) procedure followed by LSD test as post hoc comparisons of mean ($p < .05$). The software Statistica for Windows version 6 (2002) by Stasoft Inc. (Tulsa, OK) was used.

3. Results and discussion

In spite of the abundant information on physical, physicochemical and technological features of starch-based films, little is known about their bioavailability. Although films generally represent only a minor portion of the food they cover, studying their digestibility properties may provide useful information for extending uses for this type of material.

3.1. Amylose content

The amylose content is an important variable in the elaboration of starch films, since it plays a role in layer formation (Romero-Bastidas et al., 2005), although in plasticized films the final characteristics are mainly influenced by plasticizer/amylopectin interactions (Mali, Grossman, García, Martino, & Zaritzky, 2006). Table 1 summarizes the apparent amylose content assessed by an iodine-colorimetric method and a chromatography-based method. Values determined by the two procedures were in good agreement, although significant differences ($p < .05$) were

Table 1

Amylose content of native starch preparations assessed by chromatographic and colorimetric methods

Starch	Amylose (%)	
	Gel filtration	Colorimetric
Maize	$20 \pm 2^{a,b}$	$21 \pm 3^{b,c}$
Potato	18 ± 1^a	$21 \pm 2^{b,c}$
Banana	46 ± 2^e	40 ± 3^f
Sagu	$23 \pm 1^{c,d}$	$22 \pm 3^{b,c}$

Values are mean of three replicates. Means sharing superscript letters are not significantly different ($p < .05$).

observed between the two estimates in banana and potato starches.

Banana starch showed the highest amylose content (40–46%); this is in accordance with data reported by Waliszewski, Aparicio, Bello, and Monroy (2003) who found an amylose content of 40.7% for this starch. Sagu starch exhibited 22–23% amylose, values that resemble those reported by Moorthy, Andersson, Eliasson, Santacruz, and Ruales (2006) and Santacruz, Koch, Svensson, Ruales, and Eliasson (2002). Amylose content in the maize sample (20–21%) was near to that reported by Buléon, Colonna, Planchot, and Ball (1998) for this starch (25%). Finally, potato starch had the lowest amylose content (18–21%), which is similar to the amylose level recorded in wild potato starch (Buléon et al., 1998).

3.2. Available starch (AS)

Available starch (AS) contents of native starches and corresponding films are presented in Table 2. With the exception of banana starch, all samples exhibited AS contents above 91% (d.m.b.) which is indicative of satisfactory purity levels (Tovar, Melito, Herrera, Rascón, & Pérez, 2002). The lower AS value recorded for banana starch

Table 2

Potentially available (AS), total resistant (RS) and retrograded resistant (RRS) starch content of native starches and derived films

Sample	AS (%)	RS (%)	RRS (%)
Maize starch	93.02 ± 0.83^a	0.44 ± 0.15^a	0.09 ± 0.03^a
Potato starch	95.27 ± 0.83^b	45.56 ± 1.18^b	0.25 ± 0.09^b
Banana starch	86.80 ± 0.79^c	44.01 ± 1.22^b	0.91 ± 0.09^b
Sagu starch	91.61 ± 0.91^d	34.47 ± 1.17^c	1.01 ± 0.26^c
Maize starch film g	59.87 ± 0.99^g	5.35 ± 0.06^d	1.63 ± 0.02^d
Potato starch film g	60.69 ± 0.69^g	9.35 ± 0.49^c	2.01 ± 0.05^d
Banana starch film g	56.22 ± 1.36^e	7.35 ± 0.17^f	2.05 ± 0.03^d
Sagu starch film g	58.35 ± 1.47^f	9.91 ± 0.39^g	1.68 ± 0.01^d
Maize starch film ng	93.23 ± 1.38^a	16.35 ± 0.26^h	3.37 ± 0.03^c
Potato starch film ng	95.05 ± 1.13^b	15.93 ± 0.27^h	2.99 ± 0.05^c
Banana starch film ng	87.76 ± 0.31^c	15.22 ± 0.13^i	3.35 ± 0.04^c
Sagu starch film ng	92.02 ± 0.74^d	16.49 ± 0.21^h	3.48 ± 0.03^c

Values are mean of three replicates (dry matter basis).

Means in columns not sharing superscript letters are significantly different ($p < .05$).

“g” indicates films prepared with glycerol; “ng” indicates films without glycerol.

(86.8%), however, is well above that reported for the banana starch preparation obtained by González-Soto, Agama-Acevedo, Solorza-Feria, Rendón-Villalobos, and Bello-Pérez (2004), which was only around 80%. Such a behavior of banana starch may reflect the presence of higher levels of non-starch components in these preparations (Bello-Pérez, Sayago-Ayardi, Méndez-Montealvo, & Tovar, 2004; Pérez, Lares, & González, 1997). No important differences were noticed among the AS contents in maize, potato and sagu native starches. Laurentin, Cárdenas, Ruales, Pérez, and Tovar (2003) found AS levels of 97% for maize starch and of 94% for sagu starch.

The preparation of starch films using glycerol as plasticizer resulted in a remarkable decrease in the AS content. Nonetheless, when AS was calculated after correction by the actual proportion of native starch in the film-forming mixture, such a difference was ruled out (data not shown). Hence, the apparently lowered AS contents may be explained by the dilution caused by glycerol in the films, as reported by Vergara-Valencia et al. (2007) and Bello-Pérez et al. (2004) in cookies with added mango dietary fiber concentrate and in banana starch cookies, respectively.

The AS contents of films prepared in the absence of glycerol did not differ ($p < .05$) from those recorded in the parental native starches (Table 2). This result corroborates the suggested glycerol diluting effect on AS.

3.3. Resistant starch (RS)

Three of the studied starches exhibited high total resistant starch (RS) content (Table 2). The greatest values were recorded in native banana ($44.01\% \pm 1.22\%$) and potato ($45.56\% \pm 1.18\%$) starches, followed by sagu starch ($34.47\% \pm 1.17\%$). This remarkable resistance to enzymatic digestion is associated with starches whose granular crystalline organization corresponds to the B-type X-ray diffraction pattern (Englyst et al., 1992). As a matter of fact, this is the diffraction pattern reported for sagu (Gallant, Bouchet, Buléon, & Pérez, 1992; Hung & Morita, 2005; Santacruz et al., 2002; Thitipraphunkul, Uttapap, Piyachomkwan, & Takeda, 2003), potato (Gallant et al., 1992) and banana (Faisant et al., 1995) starches. It should be noted, however, that for starches from some banana varieties the A pattern has been observed (Bello-Pérez, Agama-Acevedo, Sayago-Ayardi, Moreno-Damian, & Figueroa, 2000), and more recently C-type was determined in plantain starch (Millán-Testa, Méndez-Montealvo, Ottenhof, Farhat, & Bello-Pérez, 2005).

Contrasting with the other three samples, the RS content in native maize starch was only 0.44%, an observation that is in accordance with the A-type X-ray diffraction pattern described for this starch (Gallant et al., 1992), a feature related to relatively easy digestion (Englyst et al., 1992).

Films containing glycerol showed significantly different ($p < .05$) RS contents than their parental starches. An

important decrease was noticed in potato ($9.35\% \pm 0.49\%$), banana ($7.35\% \pm 0.17\%$) and sagu ($9.91\% \pm 0.39\%$) starches. These changes are related to the disruption of the granular structure by the gelatinization process occurring during film preparation. Such a structural change was corroborated by polarized light microscopy, where native starches exhibited granules with the characteristic Maltese crosses, which completely disappeared in the films (results not shown). Thus, the absence of granular structure in potato, sagu and banana starch films made them more easily digested than the corresponding native preparations, which show a B-type X-ray diffraction pattern. Interestingly, RS content in the maize starch-based film was higher ($5.35\% \pm 0.06\%$) than in the original starch (A-type). Among reasons for this increased RS content, the possible formation of important amounts of retrograded resistant starch (RS3) during film preparation will be discussed later.

A similar behavior was exhibited by the films prepared without glycerol, even though RS values were significantly ($p < .05$) higher than in the glycerol-containing films. However, when the film RS content is calculated on a starch basis, these differences are ruled out for potato, sagu and banana, indicating that they are consequence of the previously mentioned diluting effect of the plasticizer. For maize starch, however, differences in RS contents between both types of films could not be explained by the glycerol dilution effect only. The particular X-ray diffraction pattern characteristic of this native cereal starch (A-type) may have to do with its dissimilar behavior regarding RS changes due to the film preparation protocol, a possibility that deserves further investigation.

3.4. Retrograded resistant starch (RRS)

All samples exhibited lower RRS than RS contents (Table 2). This is not surprising, since the Saura-Calixto et al. (1993) method used for assessing RRS reports only part of the indigestible starch occurring in the sample, i.e. RS3 (Tovar, 2001), whereas the method proposed by Goñi et al. (1996) includes the sum of physically inaccessible starch (RS1), ungelatinized indigestible granules (RS2) and retrograded resistant fractions (RS3).

RRS contents in the native starches were significantly lower than in corresponding films ($p < .05$), regardless of the presence of glycerol (Table 2). Such a result indicates that retrogradation occurred upon cool drying of the film-forming starch paste, whereas the starch isolation procedure involved no heating/cooling treatment, thus preventing eventual gelatinization and retrogradation. The influence of heating/cooling steps on RRS formation has been reported for several cereal, legume and root starches (Tovar et al., 2002).

RRS contents ranged between 1.63% and 2.05% in the glycerol-added films, although differences were not significant ($p < .05$). Higher levels were recorded in starch films prepared without plasticizer. Again, the lower RRS content

of glycerol-added films might reflect the polyol diluting effect. Since no statistical difference in RRS ($p < .05$) was recorded among the glycerol-free films (Table 2), it can be concluded that the retrogradation tendencies of the studied starches are similar. This is noteworthy, since banana starch is richer in amylose than the other three preparations (Table 1). It should be kept in mind, however, that starch depolymerization occurring during film-making (see below) may affect the retrogradation pattern.

Taken together, data collected on RS and RRS contents in films prepared from maize, banana, sagu and potato starches indicate the presence of crystalline (retrograded) indigestible fractions. In addition, other types of mechanisms reducing the film availability to enzymatic degradation appear to be involved. Limited solubility of films, for instance, may generate enzyme resistance due to physical inaccessibility.

3.5. Hydrolysis rate

The course of the *in vitro* α -amylolysis reaction for native starches and films prepared with glycerol and without glycerol is shown in Fig. 1. Hydrolysis values did not differ ($p < .05$) among raw native starches throughout the enzymatic reaction (5–60 min). Initial hydrolysis indices (5 min) between 0.2% and 3.5% and final (60 min) values of 1.5–9.9% min were reported by Laurentin et al. (2003) for different native raw starches, results that resemble those found in the present work. The boiling-induced gelatinization of native starches (Fig. 1a) increased significantly the hydrolysis values along the enzymatic assay; this is consequence of the granular structure destruction due to gelatinization. A particularly high rate of hydrolysis was found in the banana starch, which reached more than 90% hydrolysis after 60-min reaction; such a behavior is different from that reported by Bello-Pérez et al. (2004). These authors recorded approximately 80% digestion after 60 min incubation with pancreatic amylase. Differences in the *in vitro* α -amylolysis behavior of gelatinized banana starch might reflect the use of different banana genotypes/varieties.

Starch hydrolysis rates of both types of films (Fig. 1b and c) were higher than for the corresponding raw native starches, a fact that relates to the granular destruction associated with film preparation. No major differences were observed among the various glycerol-added starch films, even if the banana sample exhibited the greatest ($p < .05$) hydrolysis values at both initial (5 min) and final (60 min) reaction times. Nonetheless, when films were boiled before the enzymatic digestion, significant differences among samples became evident. In fact, boiled films showed greater hydrolysis indices all through the hydrolysis assay. Values ranging between 74.47% and 80.89% were recorded for boiled glycerol-containing samples and between 57.26% and 66.74% for the original glycerol-added films at 60 min reaction time. This suggests that, in spite of the lack of birefringence checked by light microscopy, native crystallite dissociation was not complete during the film-making

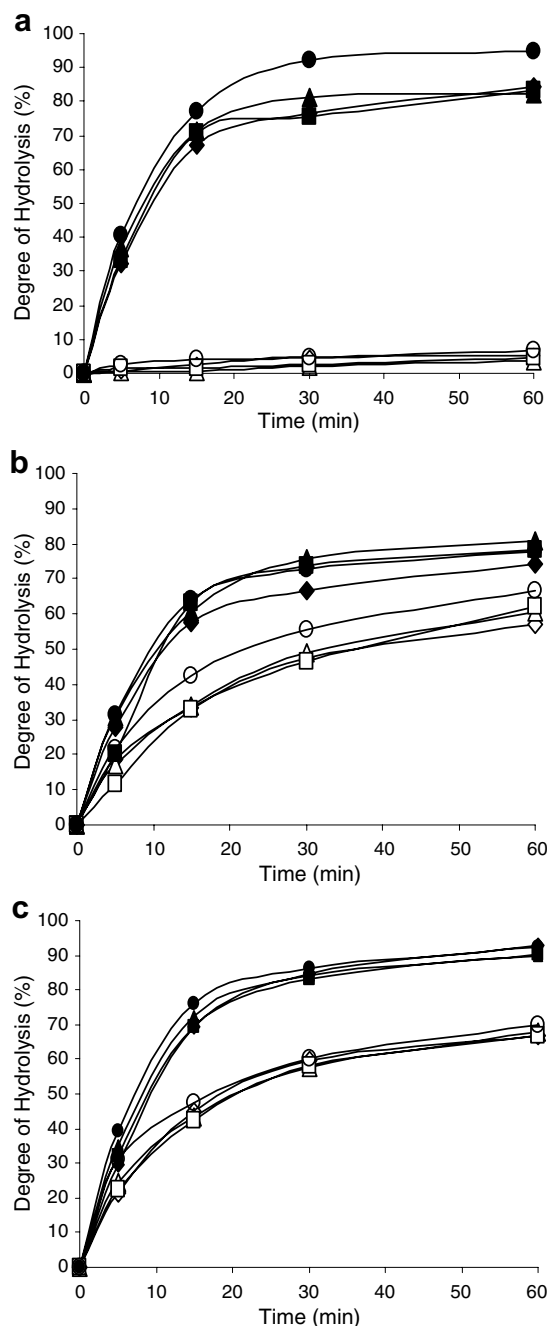


Fig. 1. *In vitro* α -amylolysis of native starches and derived films prepared with or without glycerol. (a) Parental starches; (b) starch-based films containing glycerol; (c) starch-based films without glycerol. (◆) Maize samples with pre-hydrolysis heat treatment; (◇) maize samples without pre-hydrolysis heat treatment; (▲) sagu samples with pre-hydrolysis heat treatment; (△) sagu samples without pre-hydrolysis heat treatment; (■) potato samples with pre-hydrolysis heat treatment; (□) potato samples without pre-hydrolysis heat treatment; (●) banana samples with pre-hydrolysis heat treatment; (○) banana samples without pre-hydrolysis heat treatment. Pre-hydrolysis heating was performed at 98 °C for 20 min.

ing procedure, a hypothesis that is also supported by the markedly higher RS values compared to RRS. A similar tendency was found for the α -amylolysis behavior of films prepared without glycerol (Fig. 1c); however, it is noticeable that the uncooked films were more easily hydrolyzed

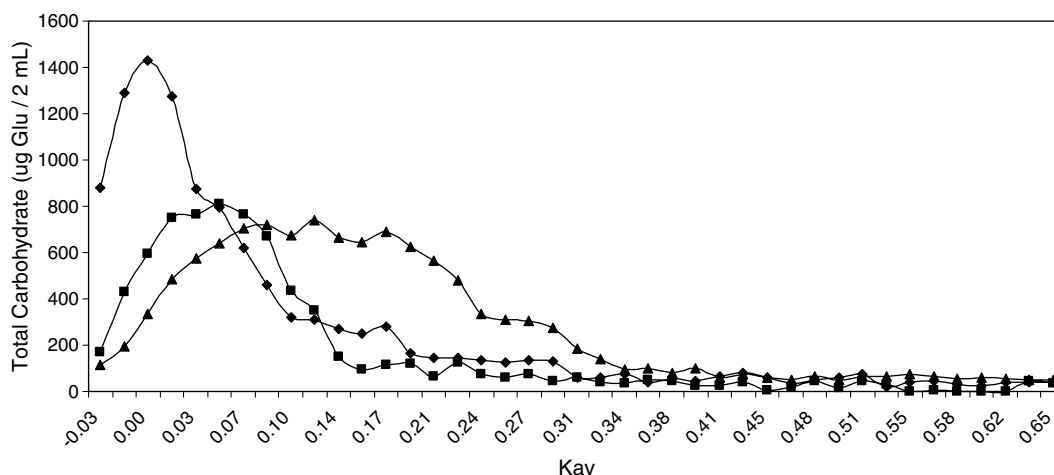


Fig. 2. Gel filtration elution profile of potato starch and derived films. (◆) Native potato starch; (■) potato starch-based film containing glycerol; (▲) potato starch-based film without glycerol.

than their glycerol-containing counterparts, with the only exception of banana starch film. The slower starch hydrolysis recorded in the films prepared with glycerol suggests that the plasticizer affects the kinetics of starch digestion. Such a phenomenon deserves further evaluation since low starch digestion rates may promote moderate in vivo glycemic responses, an important parameter to be considered in the dietary management of diabetics (Björck et al., 1994).

It should also be noted that, except for the banana samples, the starch hydrolysis rates of boiled films prepared without glycerol were significantly higher ($p < .05$) than those recorded for the gelatinized native starches. Such a result may be due to increased availability to α -amylolytic attack as a consequence of starch depolymerization/debranching occurring during preparation of glycerol-free films, a phenomenon that is discussed later on.

3.6. Gel filtration chromatography

The chromatography elution profiles of potato starch and its film (Fig. 2) are shown as examples of the gel filtration behavior of the four studied starches and their corresponding films. Potato starch exhibited the typical chromatographic behavior of native starches, with a definite amylopectin peak ($K_{av} = 0$), followed by a more scattered elution of amylose. In the films, however, the initial (amylopectin) peak was less pronounced and slightly shifted toward greater K_{av} values, which may be interpreted as amylopectin depolymerization, a change that could also include debranching. These results are in agreement with the observations by García et al. (2000), who recorded depolymerization of both amylose and amylopectin in glycerol-containing starch films prepared by cold (alkaline) gelatinization. As it was mentioned before, depolymerized/debranched starch might be more efficiently hydrolyzed by digestive enzymes such as pancreatic amylase. It is noteworthy that the altered chromatographic behavior was more evident in the films prepared without glycerol, a tendency that should be evaluated in more detail.

4. Conclusion

The digestibility features of starch-based films differ from those of parental starches. No major changes in total starch contents were noticed after film-making. Preparation of films from type B starches led to markedly decreased enzyme resistant starch contents; the opposite behavior was observed for the product prepared from maize (A-type) starch. Film manufacture resulted in increased rates of digestion, a consequence of granule disruption during production of the film-forming starch paste. Gelatinization, however, did not seem to be complete, since complementary heat treatment produced further rises in the starch hydrolysis rate. Amylopectin depolymerization may also be involved in such digestion kinetic changes. The use of glycerol as a plasticizer affects the starch digestion rate in the films, a feature worthwhile exploring when aiming to produce low glycemic index foods.

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