PHYSICOCHEMICAL PROPERTIES AND ENZYMATIC HYDROLYSIS OF DIFFERENT STARCHES IN THE PRESENCE OF HYDROCOLLOIDS

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ABSTRACT

Hydrocolloids are largely used in food processing because of their functional properties, but scarce information is available about the direct impact of different hydrocolloids on the starch digestibility. The objective of this study was to assess the effect of different hydrocolloids on the digestibility of corn and potato starch and to establish the possible relationship between physicochemical and in vitro hydrolysis of starch. Hydrocolloids significantly affected the *in vitro* hydrolysis of starch changing the pattern of the starch fractions favoring the starch hydrolysis and increasing the rapid digestible starch fraction. The effect of hydrocolloids on the starch hydrolysis was greatly dependent on the starch origin. Guar gum was the unique hydrocolloid that combined with potato starch decreases the enzymatic hydrolysis and glycemic index of this starch. Correlations were observed between hydration, pasting and starch digestibility in corn and potato starch.

Key words: starch, corn, potato, viscosity, pasting, *in vitro* digestibility.

1. INTRODUCTION

Hydrocolloids are widely used as food additives to improve the stability and texture of host foods (Chung et al., 2007), and they may also retard the retrogradation of many cereal products (Bárcenas & Rosell, 2005; Song & Park, 2003). Hydrocolloids are frequently combined with different starches to modify their rheological and pasting properties (Bárcenas et al., 2009; Lazaridou et al., 2007) but they also modify the rheological properties of protein polymers like gluten (Rosell & Foegeding, 2007). Beyond that, gums are added in food systems to improve mouth feel and to change the viscosity of solutions due to their high polymeric nature and the interactions between polymer chains when they are dissolved or dispersed (Turabi et al., 2008). Their extensive application in food technology has been supported by a vast scientific research. However, less information is available about the hydrocolloids incidence on the starch digestibility and the possible relationship between the starch digestibility and the thermal and hydration properties of starches.

Starch digestibility in flours varied with the plant source. Cereal flours have more rapidly digestible starch than legume and tuber flours, but it was not possible to determine a clear relationship between pasting properties and digestibility of flours (Liu et al., 2006). In fact, Chung et al. (2007) investigated the effect of various hydrocolloids on digestibility of cooked rice, observing that the enzymatic digestion pattern changes in the presence of hydrocolloids. However, no clear trend could be established because the global effect on the starch digestion fractions was largely dependent on the hydrocolloid type; even the glycemic index trend varied greatly with the hydrocolloid type.

Most hydrocolloids are readily soluble in water but rarely digested in human upper intestines (Edwards & Parrett, 1996; Hoefler, 2004), thus providing the same physiological response as dietary fibers (Chung et al., 2007). The functionality of the dietary fiber is attributed to their physico-chemical properties like water holding capacity, swelling, rheological behavior (Rosell et al., 2009) and also to their susceptibility to bacterial degradation or fermentation. In

60 fact, beneficial healthy effect exerted by new fiber sources has been early associated to their
61 viscosity, namely more viscous substances are more effective in decreasing postprandial
62 glucose and insulin concentrations (Jenkins et al., 1978). In healthy individuals viscous
63 polysaccharides can bring benefit because they seem to prolong the absorptive period and
64 moderate the level of nutrients in blood during the interdigestive period (Goñi et al., 2002).
65 Dartois et al. (2010) mentioned that the physiological action of hydrocolloids in the upper gut
66 could be related to their ability to produce high viscosity in the gut lumen, thereby affecting
67 the nutrient absorption and postprandial plasma nutrient levels. Although no clear relationship
68 has been found between pasting properties and digestibility of cereal, legume and tuber flours,
69 it has been suggested that the viscosity might be an important parameter for the indication of
70 starch digestibility in processed foods (Liu et al., 2006). Despite the extensive use of starch-
71 hydrocolloids blends in food technology, there is scarce information about the impact of their
72 interaction in the starch digestibility.
73 The objective of this study was to investigate the possible interference of different
74 hydrocolloids at various levels on the *in vitro* enzymatic hydrolysis of two different starches
75 (corn and potato starches) and to establish the possible correlation between the hydration and
76 pasting properties and the *in vitro* digestibility of corn and potato starches.
77

78 **2. MATERIALS AND METHODS**

79 **2.1 MATERIALS**

80 Commercial corn starch was provided by Huici Leidan SA (Navarra, Spain) and commercial
81 potato starch was supplied by Epsa Aditivos Alimentarios (Valencia, Spain). Hydrocolloids
82 included high methoxylated pectin (GENU® pectin 150 USA-SAG type BA-KING from
83 CPKelco), guar gum (Guar gum - 3500 from EPSA, Spain) carboxymethylcellulose food
84 grade (CMC) (Methocel A4M from Dow Wolff Cellulosics, France), xanthan gum food grade
85 (Jungbunzlauer, Austria) and hydroxypropylmethylcellulose (HPMC) (Methocel K4M from

86 Dow Wolff Cellulosics, France). Resistant starch assay kit GOPOD (Cat. No. K-RSTAR)
87 was purchased from Megazyme (Megazyme International Ireland Ltd., Bray, Ireland).
88
89 **2.2 Methods**
90 2.2.1 Hydration properties
91 The effect of hydrocolloids on the hydration properties were determined by mixing the
92 starches with the hydrocolloids at four levels (1, 2, 3 and 4%, w/w starch basis), and also
93 hydration properties of the starches were assessed in the absence of hydrocolloid. Levels of
94 hydrocolloids were selected on the basis of the common range used in bakery applications
95 included gluten and gluten free foodstuff (Bárcenas et al., 2005; Marco & Rosell, 2008). The
96 swelling volume was determined following the method reported by Nelson (2001) with slight
97 modification. Briefly, dried samples (0.5 g ± 0.1 mg) were placed in a graduated cylinder (100
98 ml) and mixed with distilled water (30 ml), then kept at room temperature for 24 h. The
99 swelling volume was calculated by dividing the total volume of the swollen sample by the
100 original dry weight of the sample.
101 The water holding capacity (WHC) defined as the amount of water retained by the sample
102 without being subjected to any stress was determined as described the standard method
103 (AACC, 1994). Powder samples (2 g ± 0.1 mg) were mixed with deionized water (20 ml) and
104 kept at room temperature for 24 h. WHC was expressed as grams of water retained per gram
105 of solid.
106
107 2.2.2 Pasting properties
108 The pasting properties were measured using a Rapid Viscoanalyser (RVA) (Newport
109 Scientific model 4-SA, Warriewood, Australia). The viscosity parameters were recorded in cP
110 units (1 cP=1 mPa s^{-1}). The 2.5 g (14% moisture basis) sample was dispersed in 25 ml
111 distilled water, mixed in the RVA aluminum sample bin and measured. The condition settings

were sample equilibration at 50 ºC for 1 min, heating from 50 to 95 ºC for 3.5 min, holding at 95 ºC for 5 min, cooling down to 50 ºC for 3.5 min and then holding at 50 ºC for 4 min. Paddle speed was 960 rpm for first 10 s, then set at 160 rpm for running the analysis. Pasting parameters included peak time (min), peak viscosity (cP), final viscosity (cP), breakdown (cP), setback (cP), and pasting temperature (ºC), which were determined from the recorded curve. The reported values are means of duplicate measurements. Pastes obtained from the RVA were freeze dried and kept at 4ºC for further starch hydrolysis assays.

2.2.3 In vitro starch digestibility and expected glycemic index

Digestibility of starches and starch-hydrocolloids blends was determined in the freeze dried pastes obtained from the RVA. Powder sample (100 mg) was incubated with porcine pancreatic α-amylase (10 mg/ml) and amyloglucosidase (3.3 U/ml) in 4 ml of 0.1 M sodium maleate buffer (pH 6.0) in a shaking water bath at 37 ºC (0.5-16 h). Aliquots of 200µl were withdrawn during the incubation period. Aliquots were kept in a boiling water bath for five minutes to finalize the enzymatic reaction, then 200µl of ethanol (96%) was added and the sample was centrifuged for five minutes at 10,000 x g and 4ºC. The pellet was washed twice with 50% ethanol (100µl) and the supernatants were pooled together and kept at 4ºC for further glucose determination.

The remnant starch after 16 hour hydrolysis was solubilized with 2 ml of 2 M KOH using a Virtis homogenizer (3 x 10 s strokes at 16000 rpm). The homogenate was diluted with 8 ml 1.2 M sodium acetate pH 3.8 and incubated with 100µl amyloglucosidase (330 U) at 50ºC for 30 minutes in a water shaking bath. After centrifuging at 2,000 x g for 10 min, supernatant were kept for glucose determination.

The glucose content was measured using a glucose oxidase-peroxidase (GOPOD) kit. The absorbance was measured using a microplate reader (Spectramax 190, Molecular Devices) at

510nm. In all cases four replicates were assayed for each experimental point. Starch was calculated as glucose (mg) x 0.9.

According to the hydrolysis rate of starch, three different fractions were quantified as suggested Englyst et al. (1992). Rapidly digestible starch (RDS) was referred to the percentage of total starch that was hydrolyzed within 30 min of incubation, slowly digestible starch (SDS) was the percentage of total starch hydrolyzed within 30 and 120 min, and resistant starch (RS) was the starch remaining unhydrolyzed after 16 h of incubation. The percentage of total starch hydrolyzed at 90 minutes (H_{90}) was also calculated.

The *in vitro* digestion kinetics was calculated in accordance with the procedure established by Goñi et al. (1997). A nonlinear model following the equation [$C = C_{\infty}(1 - e^{-kt})$] was applied to describe the kinetics of starch hydrolysis, where C was the concentration at t time, C_{∞} was the equilibrium concentration or maximum hydrolysis extent, k was the kinetic constant and t was the time chosen. The hydrolysis index (HI) was obtained by dividing the area under the hydrolysis curve (0–180 min) of the sample by the area of a standard material (white bread) over the same period of time. The expected glycemic index (eGI) was calculated using the equation described by Grandfeldt et al. (1992): eGI = 8.198 + 0.862HI.

2.2.4 Statistical analysis

Experimental data were statistically analyzed by using Statgraphics V.7.1 program (Bitstream, Cambridge, MN) to determine significant differences among them. When ANOVA indicated significant F values, multiple sample comparison was also performed and Fisher's least significant difference (LSD) procedure was used to discriminate among the means, and correlation matrix was carried out by the Pearson-product moment to significant $p<0.05$.

3. RESULTS AND DISCUSSION

3.1 Effect of hydrocolloids on physicochemical properties of starches

163 It has been previously reported that hydrocolloids alter the hydration and pasting behavior of
164 starch granules, and the extent of the modification is greatly dependent on the type and level
165 of hydrocolloid and the starch origin. Owing to the variation of results reported, the impact of
166 the specific hydrocolloids used in this study in some hydration properties and the pasting
167 behavior of two different starches were determined to establish correlations between those
168 properties and the starch digestibility. Two different starches were selected to have better
169 understanding of the incidence of hydrocolloids on starch hydrolysis.
170 Hydration properties of corn and potato starches in the presence of diverse hydrocolloids
171 added at different levels (1, 2, 3 and 4%) are shown in Table 1. Swelling values were
172 comprised within the range 7.5 and 21 ml/g. Hydrocolloids affected in different extent the
173 starch swelling and the effect was dependent on the starch origin and the hydrocolloid level.
174 Guar gum and xanthan gum showed the highest effect on both starches, inducing a significant
175 increase of this parameter, although in the case of potato starch guar gum levels higher than
176 2% were required. All hydrocolloids tested increased the swelling of corn starch, but levels
177 higher than 1% of CMC or 2% of pectin and HPMC were needed for producing a significant
178 ($p<0.05$) increase. No significant effect on potato starch swelling was promoted by pectin or
179 the cellulose derivatives (CMC and HPMC). It is generally assumed that the hydrophilic
180 nature of the hydrocolloids increase the water retention, and in consequence hydrocolloids can
181 have significant influence on starch swelling (Kulicke et al., 1996; Rojas et al., 1999). Song et
182 al. (2006) reported that hydrocolloids (gellan gum, guar gum, xanthan gum and Arabic gum)
183 reduced rice starch swelling because of the osmotic pressure generated within the continuous
184 hydrocolloid phase hindered the water accessibility to the starch granules. Nevertheless, that
185 tendency was reversed at higher hydrocolloid concentration (0.1%) because the settling of
186 swollen granules could be somewhat impeded by the high viscosity. However, the chemical
187 structure and shape of the hydrocolloids must play an essential role (Rosell et al., 2009), being
188 responsible of the different trend observed with each pair hydrocolloid-starch.

189 In general, hydrocolloids tended to increase the water holding capacity of the studied starches
190 (Table 1), although some exceptions were detected. Pectin did not affect the WHC of starches
191 at any level, and CMC did not influence the WHC of potato starch. Hydrocolloids effect was
192 more noticeable on corn starch than in potato starch.
193
194 The effect of hydrocolloids on the pasting properties of corn and potato starch is shown in
195 Figure 1. Potato and corn starch differed in the pasting behaviour, observing much higher
196 viscosities during heating and cooling in the case of potato starch, which agree with previous
197 findings (Liu et al., 2006). Hydrocolloids affected the pasting properties of both corn and
198 potato starches, although the effect was highly dependent on the hydrocolloid nature. Great
199 dissimilarity was encountered in the heating-cooling cycle depending on the starch origin,
200 observing that hydrocolloids affected mainly gelatinization process of potato starch, whereas
201 gelatinization and gelification of corn starch was modified in the presence of hydrocolloids
202 (Figure 1). Table 1 shows the parameters that define the pasting and gelling behavior of corn
203 and potato starch in the presence of different levels of hydrocolloids. No clear trend was
204 observed with the hydrocolloid level added to the starches.
205 The peak time or time to reach the maximum viscosity was significantly ($p<0.05$) increased
206 by pectin and xanthan; and guar gum only augmented the peak time when added to corn
207 starch. Therefore, longer cooking time was required for corn and potato starch gelatinization
208 in the presence of those hydrocolloids, likely due to an stabilizing effect of the hydrocolloids
209 on the starch granules, since a positive correlation was observed between peak time and the
210 WHC for both starches ($r = 0.8671$, $p<0.001$ for corn starch and $r = 0.582$, $p<0.001$ for potato
211 starch).
212 Pectin induced a significant ($p<0.05$) decrease of the peak viscosity, breakdown, final
213 viscosity and setback of the potato starch, but only decrease the breakdown (when added up to
214 2%) and setback of corn starch. The addition of guar gum to potato starch resulted in higher

values for peak viscosity, breakdown, final viscosity and setback, but only a noticeable increase in peak viscosity and final viscosity was induced in corn starch (Table 2). Song et al. (2006) and lately Rosell et al. (2010) found similar results when adding guar gum to rice starch, increasing the viscosity of rice starch during heating and cooling and the effect was dependent on the gum concentration. Guar gum promotes an increase of the capacity of the starch granules to swell, likely due to the inhibition of starch components from leaching out the granule compounds into the continuous phase of pastes during gelatinization, which resulted in viscous systems, as suggested Dartois et al. (2010).

Conversely, the addition of xanthan to corn and potato starches resulted in a decrease of the peak viscosity, breakdown and setback; moreover a decrease in the final viscosity of potato starch was observed. The opposite behavior has been described when xanthan gum up to levels of 0.2% was added to rice starch (Song et al., 2006). Likely the higher levels of hydrocolloid used in the present study are responsible of the reverse effect since the starch-hydrocolloid network is highly dependent on the starch-hydrocolloid ratio (Kulicke et al., 1996; Rosell et al., 2010). Cellulose derivatives (CMC and HPMC) caused minor effect on the pasting properties of the studied starches, which agree with results of Bárcenas et al. (2009) obtained with wheat starch. CMC decreased the breakdown and setback of corn starch, whereas HPMC only decreased the breakdown of this starch. Regarding potato starch, CMC decreased the final viscosity and HPMC decreased the peak viscosity and the breakdown. Cellulose derivatives such as CMC and HPMC are water-soluble cellulose ethers compatible with a wide range of other food ingredients, including starches, over a wide concentration range (Techawipharat et al., 2008), probably that compatibility is responsible of the little effect observed on the pasting properties. Rojas et al. (1999) indicated that modifications which result from the addition of hydrocolloids to a starch system are complex, and these can be ascribed to polymers interactions or phase separation processes in relation to incompatibility phenomena between unlike polymers.

241 WHC and viscosity are two physicochemical properties that are normally correlated, but in
242 the case of pasting properties only a positive relationship ($r=0.4012$, $p<0.001$) was found with
243 the peak time and a negative correlation ($r=-0.2566$, $p<0.01$) with the setback. The same
244 trend has been reported by León et al. (2010).
245 The assessment of hydration and pasting properties results crucial for food technology
246 applications, but also it has been pointed out the relationship between viscosity of soluble
247 fibers and their physiological role (Jenkins et al., 1978). In the present study, only guar gum
248 induced an increase in the starch paste viscosity during heating and cooling and that effect
249 was independent on the starch origin.
250
251 **3.2 Effect of hydrocolloids on *in vitro* starch digestibility**
252 Starch can be classified into three main fractions according to their rate and extent of *in vitro*
253 digestion: rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch
254 (RS), the later is considered a fiber because it is not absorbed in the small intestine of healthy
255 individuals (Skrabanja et al., 1998). The designations rapidly digestible glucose (first 30 min)
256 and slowly digestible glucose (from 30 to 120 min) reflect the rate at which glucose (from
257 sugars and starch, including maltodextrin) becomes available for absorption in the small
258 intestine. Englyst et al. (1996) have previously shown a strong correlation between rapid
259 elevation of postprandial plasma glucose and insulin and rapidly available starch values for a
260 wide range of dry starchy foods. The possible incidence of hydrocolloids on starch hydrolysis
261 kinetics and the different starch fractions was determined in the pastes of starch-hydrocolloids
262 blends obtained after heating and cooling in the RVA.
263 The predominant fraction in corn and potato pastes was the SDS followed by RDS, and a
264 minor content of RS (Figure 2). However, that pattern changes when starches were blended
265 with different hydrocolloids, and the effect was greatly dependent on the type of starch. No
266 significant effect was observed when added different hydrocolloids levels. The effect of 2%

hydrocolloid on the *in vitro* starch hydrolysis is showed in Figure 2. Hydrocolloids blended with corn starch induced a significant increase of the RDS fraction with a concomitant decrease of the SDS fraction. Regarding the RS fraction, with the exception of xanthan and HPMC that decreased the RS content, the other tested hydrocolloids did not modify the content of RS fraction in corn starch.

The RDS fraction in the paste of potato starch increased when was blended with hydrocolloids, with the exception of guar gum that decreased that fraction. Hydrocolloids did not modify the amount of SDS fraction when added to potato starch, except in the case of pectin that induced a significant decrease. The RS values in potato starch increased in the presence of hydrocolloids, with the exception of HPMC. Therefore, hydrocolloid addition resulted in a shift between digestible and non-digestible fractions, which was dependent on the starch source. Moreover, the changes observed in the *in vitro* digestion indicated an increase of the rapid-release properties in corn starch, which was less marked in potato starch. In general, it is more desirable the SDS over the RDS, since SDS is slowly digested in the small intestine and induces gradual increase of postprandial plasma glucose and insulin levels (Jenkins et al., 1978).

Soluble dietary fiber acts like a sponge and absorbs water in the intestine; it mixes with the food to form an entangled network, and thereby slows down the rate of digestion and absorption (Dartois et al., 2010). That effect has been connected to the viscosity of certain polysaccharides because they seem to retard the absorption of nutrients and in turn their appearance in the blood system (Jenkins et al., 1978). In the present study possible correlations between pasting properties and *in vitro* starch hydrolysis were investigated. A positive relationship was found between the final viscosity and RDS ($r = 0.2943$, $p<0.05$) when hydrocolloids were blended with corn starch. In the case of potato starch RDS showed negative correlation with final viscosity ($r = -0.3112$, $p<0.05$) and breakdown ($r = -0.5426$,

293 $p<0.001$), whereas SDS was positively correlated with peak viscosity ($r=0.5966$, $p<0.001$),
294 breakdown ($r=0.5906$, $p<0.001$), final viscosity ($r=0.4215$, $p<0.01$) and setback ($r=0.4352$,
295 $p<0.01$), except for xanthan gum. Soluble cellulose derivatives (CMC and HPMC) were the
296 main responsible of those correlations. Englyst et al. (1996) reported that the breakdown of
297 solid starchy foods could predict the postprandial response *in vivo* and that SDS has limited
298 effect on the glycemic response but it is available as sugar. In addition, WHC for the potato
299 starch showed positive correlation with SDS ($r = 0.3927$, $p< 0.005$), indicating the incidence
300 of hydration on the starch hydrolysis. Likely, starch swelling enhances the accessibility of
301 digestive enzyme into the granules and thus increases the RDS content (Chung et al., 2008).
302 The same authors reported that gelatinized corn starch hydrolyses more readily than its prime
303 type, yielding higher RDS content and lower SDS and RS contents.
304
305 **3.4 Effect of hydrocolloids on hydrolysis kinetics and estimated glycemic index**
306 Primary and secondary parameters derived from the *in vitro* digestion of starches blended
307 with different hydrocolloids are listed in Table 2. Those parameters included equilibrium
308 concentration of hydrolyzed starch (C_∞), kinetic constant (k), of total starch hydrolysis at 90
309 min (H_{90}), area under the hydrolysis curve after 180 minutes (AUC 180), hydrolysis index
310 (HI) and estimated glycemic index (eGI).
311 The kinetic constant, indicative of the hydrolysis rate in the early stage, increased in the
312 presence of hydrocolloids in both starches, the unique exception was the blend potato starch
313 and guar gum at levels higher than 1%. The slower rate of potato starch hydrolysis in the
314 presence of guar gum may be attributed to its high capacity to increase the viscosity of the
315 matrix, which affects the sugars and enzymes diffusion and also the enzymatic activity due to
316 enlargement of fully hydrated galactomannan chains (Dartois et al., 2010). Likely, the trend
317 change observed when adding increasing levels of guar gum could be attributed to the change
318 in hydrocolloid-starch interaction. It has been described that the gels obtained in the presence

319 of hydrocolloids showed diverse rheological behavior depending on the hydrocolloid
320 concentration (Rosell et al., 2011). In fact, low levels of guar (<0.5%) led to composite
321 network structures with less number of junction zones among the gum and rice starch, but
322 higher levels favor the formation of a network structure with less gel-forming junction zones
323 with the starch and more entanglements between the hydrocolloid chains (Kulicke et al.,
324 1996), conducting to phase separation (Alloncle & Doublier, 1991). Therefore, a plausible
325 explanation for the different trend observed at guar gum levels higher than 1% would be the
326 phase separation. Present results also suggest that high levels of guar gum will be required for
327 obtaining phase separation in potato starch gels.
328 The increase of the hydrolysis rate of corn and potato starches induced by hydrocolloids
329 agrees with the increase of RDS above described. In general, hydrocolloids increase the early
330 digestion of granular starch, which might be related to the enhanced swelling of starch
331 granules as has been suggested for chemically modified starches (Chung et al., 2008).
332 Concerning the possible relationship with physicochemical properties, again no general trend
333 could be established for both types of starches. Correlations were only found for potato starch.
334 The kinetic constant of potato starch showed negative correlation with peak viscosity ($r=-0.5416$, $p<0.001$), breakdown ($r=-0.5416$, $p<0.001$), setback ($r=-0.3422$, $p<0.05$) and WHC
336 ($r=-0.3161$, $p<0.05$). This result agrees with previous observation that viscous solutions
337 influence the kinetic of the enzymatic hydrolysis (Dartois et al., 2010).
338
339 The maximum hydrolysis, C_∞, of corn starch paste was significantly higher than that of potato
340 starch paste. Hydrocolloids significantly affected C_∞, but no general trend was observed with
341 the level of hydrocolloid. The C_∞ values of corn starch were significantly enhanced by adding
342 CMC, but in potato starch that effect was observed with guar gum and pectin. Conversely, the
343 presence of xanthan decreased C_∞ in the case of corn starch and only when added 3% level to
344 potato. Therefore, diverse changes were observed depending on the hydrocolloid type. Some

hydrocolloids retard the amylose retrogradation due to hydrocolloids-amylose interaction (Rojas et al., 1999) and that could facilitate the enzyme attack, but at the same time hydrocolloids could retard the enzymatic hydrolysis by coating the surface of the starch granules, acting as a physical barrier to either the enzyme attack or the release of hydrolysis products (Chung et al., 2007; Dartois et al., 2010). Therefore, it seems that the resulting effect of hydrocolloids on starch digestibility is rather dependent on the starch-hydrocolloid interaction, which could fall either in the composite network category or in two phase separation due to the chains rearrangement after heating and cooling (Rosell et al., 2010).

The maximum hydrolysis was positively correlated in corn starch with final viscosity ($r = 0.3471$, $p<0.014$), whereas the C_∞ in potato starch showed a negative correlation ($r = -0.4783$, $p<0.001$).

The H_{90} (percentage of total starch hydrolysis at 90 min) is another parameter related to starch digestibility. The H_{90} of corn and potato starches combined with hydrocolloids were significantly higher compared with those obtained for the individual starches, except for potato starch blended with guar gum. Corn starch showed the highest increase of H_{90} above 75% in the following order CMC > HPMC > guar gum > xanthan > pectin (Table 2). A factor of interest is the time of transit through the colon that determines the duration of the contact with the bacterial enzymes, and the dietary fibre components that limit the extent of its decomposition (30-90% polysaccharides, mainly hemicelluloses and pectin) and the main effect in the small intestine is associated with the viscous polysaccharides, such as pectins and gums, which decrease the assimilation of nutrients, while the insoluble components do not affect in great extent (Rodrígues et al., 2006). It seems that the starch susceptibility to enzymatic hydrolysis increases with the swelling ability, but also starch origin and hydrocolloid nature affected that behaviour. Goñi et al., (2002) reported that the nature of polysaccharide determines its physico-chemical behaviour and this may affect the rate of digestion of carbohydrates and absorption of sugars in the small intestine. This fact was

371 confirmed with some correlations of H_{90} values with physicochemical properties. H_{90} of corn
372 starch showed a positive correlation with some pasting and hydration parameters as peak
373 viscosity ($r = 0.4154$, $p<0.01$), final viscosity ($r = 0.3943$, $p<0.01$), swelling ($r = 0.4775$,
374 $p<0.001$) and WHC ($r = 0.3501$, $p<0.05$), whereas for potato starch it was found a negative
375 correlation with peak viscosity ($r = -0.4630$, $p<0.001$) and breakdown ($r = -0.4689$, $p<0.001$).
376 A comprehensive parameter for the starch digestibility (Figure 3) is the total area under the
377 hydrolysis curve [AUC ($mg_{glucose}/g_{sample}$) x min] relating the glucose release over a hydrolysis
378 period of 180 min (Goñi et al., 1997). The type of starch had a significant effect on the AUC
379 180 min values ($p<0.05$), being the value for potato starch higher than that for corn starch.
380 When starches were blended with different hydrocolloids the resulting pastes of the RVA
381 showed significantly higher AUC 180 min, with the exception of the combination potato
382 starch-guar gum that decreased that parameter. Again, the high viscosity induced by this
383 hydrocolloid might form a physical barrier hindering the α-amylase access (Dartois et al.,
384 2010), and the different trend observed at 1% gum level could be the result of the absence of
385 phase separation (Kulicke et al., 1996).
386 The combination guar gum with potato starch yielded pastes that were slowly hydrolyzed, and
387 in consequence, lower glucose liberation under *in vitro* conditions was taken place, thus
388 probably the intake of potato starch blended with guar gum slows down the gastric empty and
389 reduces the rate of intestine absorption of glucose. Jenkins et al. (1978) reported that *in vivo*
390 experiments carried out with a solution of guar gum and sugar showed a reduction of the area
391 under the curve for insulin response showing positive correlation with the viscosity.
392
393 The effect of gums on the human metabolism is considered beneficial because they decrease
394 postprandial glycemia following ingestion of starchy food due to their ability to produce high
395 viscosity in the gut lumen, thereby affecting the nutrient absorption and postprandial plasma
396 nutrient levels (Dartois et al., 2010). This effect has been associated with glycaemic lowering

397 effect (Goñi et al., 2002). However, the present study showed that the effect of hydrocolloids
398 on *in vitro* starch hydrolysis was dependent on the specific starch-hydrocolloid combination,
399 with a general tendency to increase the hydrolysis rate. Likely differences observed with
400 previous results can be due to the association of starch with other macropolymers present in
401 the flours or in the products that could interact with the starch affecting the starch
402 digestibility, and having a direct consequence on the glycemic response of the carbohydrate
403 based products (Fardet et al., 2006).
404
405 The presence of hydrocolloids also modified the estimated glycemic index. The eGI values
406 increased in the presence of hydrocolloids with the exception of guar gum when added to
407 potato starch. Considering that low glycemic food are desirable to generate and moderate
408 postprandial glucose and insulin response, only the combination potato starch with guar gum
409 would be advisable.
410
411 **Conclusion**
412 The present study confirmed that pasting and hydration properties of starch are significantly
413 affected by hydrocolloids and that effect was dependent on the hydrocolloid nature and the
414 starch origin. Hydrocolloids significantly affect the *in vitro* hydrolysis of starch changing the
415 pattern of the starch fractions favouring the starch hydrolysis and increasing the RDS fraction.
416 The effect of hydrocolloids on the starch hydrolysis was dependent on the starch origin. *In*
417 *vitro* studies of starch digestibility showed that hydrocolloids accelerate the enzymatic
418 hydrolysis rate in the early stage, with the exception of the pair potato starch-guar gum. In
419 general, hydrocolloids induce a shift from slow digestible starch to rapid digestible starch.
420 Moreover, the changes observed in the *in vitro* digestion indicated an increase of the rapid-
421 release properties in corn starch, which was less marked in potato starch. The guar gum
422 decreases the enzymatic hydrolysis and glycemic index of potato starch, which is likely

423 associated to the increase of viscosity. Correlations have been established between starch
424 digestibility and physicochemical properties and they were greatly dependent on the starch
425 origin. Among the most important correlation, it should be pointed out the positive
426 correlations observed in the case of potato starch between SDS with peak viscosity,
427 breakdown, final viscosity, setback and WHC.
428
429 **Acknowledgements**
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434
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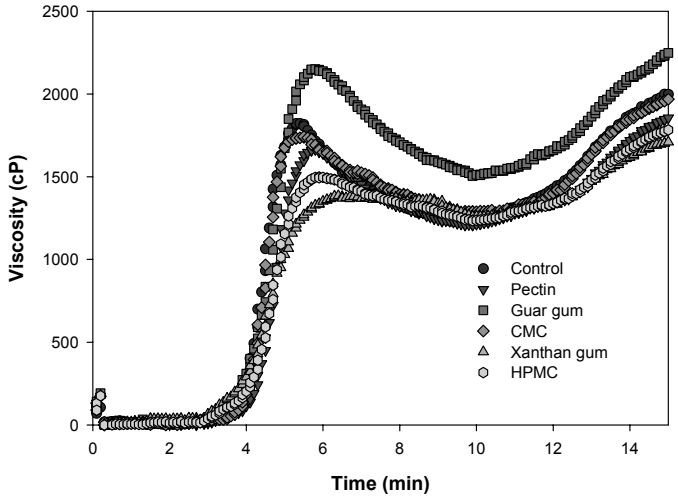
511 **FIGURE CAPTIONS**
512
513 **Figure 1**. Effect of hydrocolloids on pasting properties of starch determined by rapid
514 viscoanalyzer. A: Corn starch, B: Potato starch.
515
516 **Figure 2.** Effect of hydrocolloids (2%, w/w) on *in vitro* starch digestibility. A: Corn starch
517 (C), B: Potato starch (P). Error bars indicate standard deviation. Letters within each starch
518 fraction indicated significant differences ($p<0.05$).
519
520 **Figure 3**. Corn and potato starch hydrolysis pattern blended with 2% (w/w) of different
521 hydrocolloids. A: Corn starch (C), B: Potato starch (P).

Figure 1.

A.


Viscosity (cP)
Time (min)
Control
Pectin
Guar gum
CMC
Xanthan gum
HPMC

B.

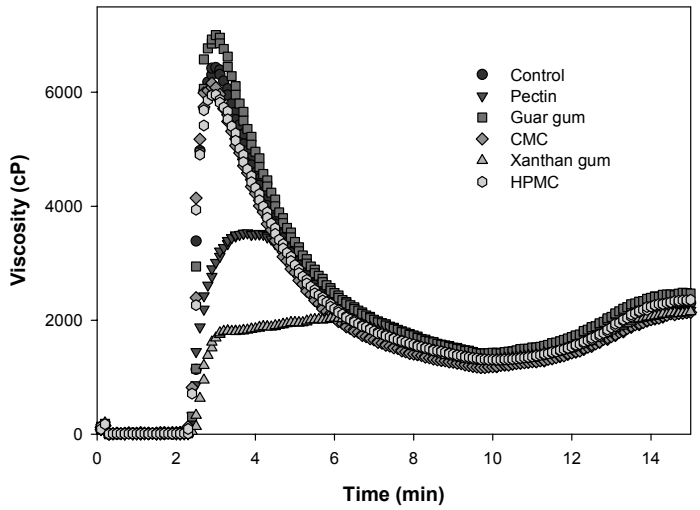
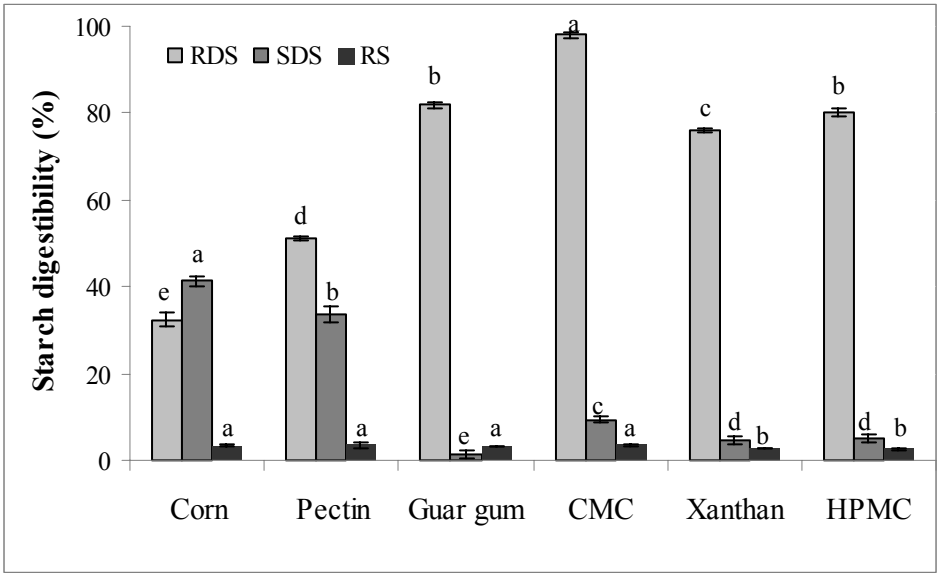

Viscosity (cP)
Time (min)
Control
Pectin
Guar gum
CMC
Xanthan gum
HPMC

Figure 2.

A.


RDS
SDS
RS
Starch digestibility (%)
100
80
60
40
20
0
Corn
Pectin
Guar gum
CMC
Xanthan
HPMC

B.

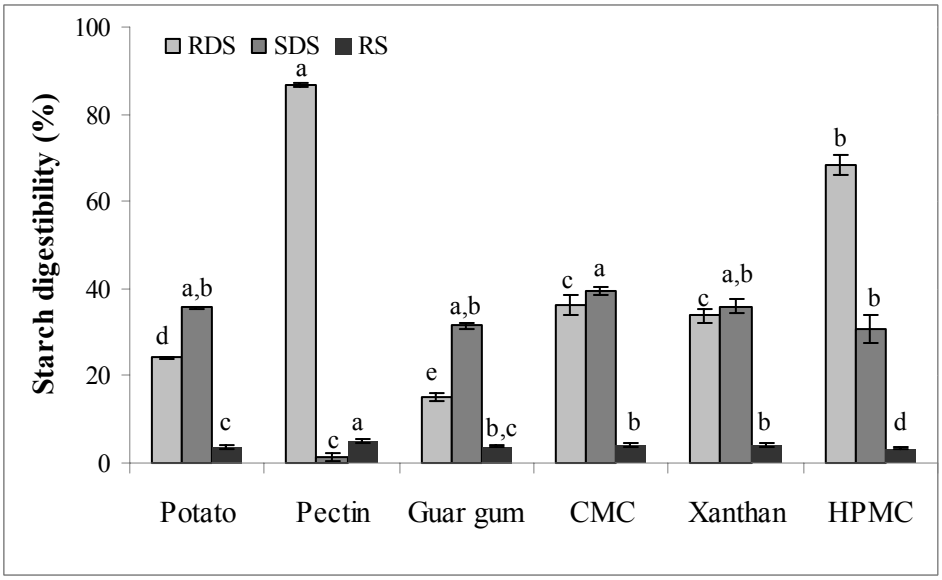
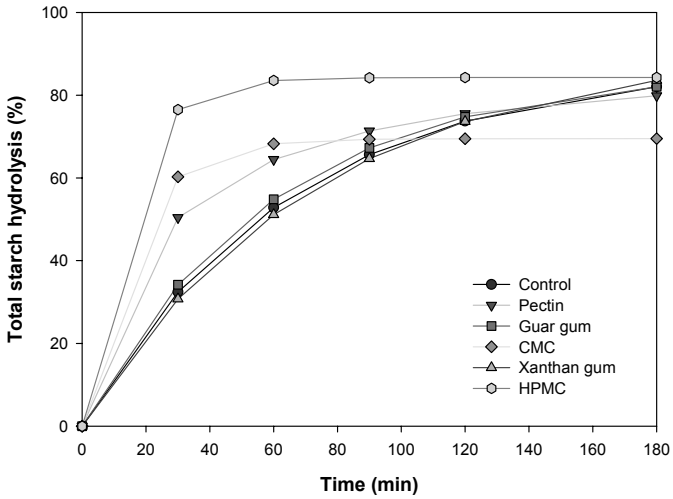
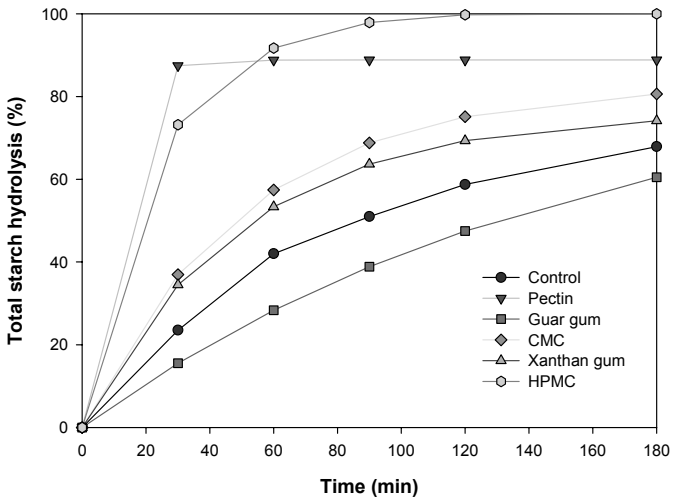

RDS
SDS
RS
Starch digestibility (%)
100
80
60
40
20
0
Potato
Pectin
Guar gum
CMC
Xanthan
HPMC

Figure 3.

A.


Total starch hydrolysis (%)
Time (min)
Control
Pectin
Guar gum
CMC
Xanthan gum
HPMC

B.


Total starch hydrolysis (%)
Time (min)
Control
Pectin
Guar gum
CMC
Xanthan gum
HPMC

541 **Table 1**. Effect of hydrocolloids on hydration and pasting properties[a]

Blends	Hydrocolloid (%)	Swelling (ml/g)	WHC (g water/g solid)	Pasting Temperature (ºC)	Peak time (min)	Peak viscosity (cP)	Breakdown (cP)	Final viscosity (cP)	Setback (cP)
corn	0	7.5 h	1.34 j	77.3 a-c	5.3 hi	1823 d	601 b-d	2143 b-f	920 a
Pectin	1	8.0 gh	1.55 h-j	81.6 a-c	5.7 d-f	1618 ef	390 hi	1961 e-h	734 c-e
	2	8.1 gh	1.7 g-j	65.5 c-e	5.8 d	1666 d-f	459 f-h	1976 d-h	769 b-d
	3	8.6 fg	1.44 ij	64.8 c-e	5.7 de	1758 de	601 b-e	1986 d-h	829 a-d
	4	8.8 fg	1.67 h-j	65.6 c-e	5.7 de	1705 d-f	546 c-f	1942 f-h	783 b-d
Guar gum	1	9.7 f	2.64 fg	83.3 ab	5.7 de	2014 bc	629 b-d	2282 a-c	898 ab
	2	14.5 c	3.41 e	63.1 de	5.8 d	2159 b	656 a-c	2366 a	863 a-c
	3	17.3 b	4.3 d	75.6 a-e	5.7 d-f	2129 b	686 ab	2324 ab	881 ab
	4	14.5 c	5.1 c	65.3 c-e	6.0 c	2367 a	763 a	2406 a	801 b-d
CMC	1	8.0 gh	1.89 h-j	79.6 a-e	5.4 g-i	1850 cd	489 e-h	2150 a-g	789 b-d
	2	8.7 fg	2.61 fg	84.2 ab	5.5 f-h	1744 de	480 f-h	2065 b-g	801 b-d
	3	10.2 e	2.9 ef	62.2 de	5.3 i	1775 de	528 e-g	2053 c-g	807 b-d
	4	11.8 e	3.31 e	75.2 a-e	5.3 g-i	1668 d-f	482 f-h	1950 e-h	764 b-d
Xanthan	1	12.8 d	4.85 cd	79.1 a-d	6.6 b	1517 fg	197 jk	1941 f-h	621 ef
	2	16.5 b	6.54 b	58.8 e	6.6 b	1407 g	153 k	1780 h	526 f
	3	21.0 a	6.72 b	58.7 e	7.0 a	1755 de	280 ij	2206 a-e	731 c-e
	4	n.d. i	9.11 a	74.9 a-e	7.0 a	1621 ef	302 ij	2092 b-g	773 b-d
HPMC	1	8.0 gh	1.72 i-k	86.4 a	5.5 g	1629 ef	424 gh	1915 gh	709 de
	2	8.3 gh	2.06 g-i	78.5 a-d	5.4 g-i	1738 de	447 f-h	2182 a-f	891 ab
	3	12.0 d	2.28 gh	68.3 b-e	5.4 g-i	1710 d-f	399 hi	2160 a-f	849 a-c
	4	12.0 d	2.53 fg	64.7 c-e	5.5 e-g	1719 d-f	380 hi	2228 a-d	889 ab
Potato	0	7.8 ef	1.29 g	60.1 bc	3.0 e	6434 b	5102 c	2227 c	894 c
Pectin	1	7.0 e	1.21 fg	66.2 ab	4.1 bc	3868 f	2566 g	2265 bc	963 a-c
	2	7.1 ef	1.27 fg	64.3 a-c	3.9 b-d	3535 fg	2338 g	2078 e-g	880 b-d
	3	7.4 ef	1.27 fg	65.3 ab	4.0 bc	3434 g	2378 g	1844 hi	789 de
	4	7.8 ef	1.3 fg	66.6 ab	4.1 bc	3713 fg	2683 g	1740 i	710 ef
Guar gum	1	6.8 e	2.43 c-f	67.6 ab	3.1 de	6926 a	5522 b	2357 ab	953 a-c
	2	7.0 e	3.64 bc	67.2 ab	3.1 de	7176 a	5760 ab	2420 a	1004 a
	3	18.0 b	4.89 a	66.8 ab	3.0 de	7250 a	5800 ab	2436 a	986 ab
	4	18.5 ab	4.71 ab	62.1 a-c	3.1 de	7333	5935 a	2415 a	1017 a

CMC	1	7.0	e	1.55	e-g	62.6	a-c	2.9	e	6350	b-d	5060	c	2182	c-e	892	b-d
	2	7.0	e	1.69	e-g	58.2	a-c	3.0	e	6176	b-d	5016	cd	2094	d-f	933	a-c
	3	6.9	e	1.94	d-g	66.4	ab	2.8	e	5956	de	4870	c-e	1954	gh	868	cd
	4	7.0	e	1.91	e-g	65.8	ab	3.0	de	5731	e	4567	ef	1970	f-h	806	de
Xanthan	1	14.0	d	1.38	e-g	66.7	ab	3.3	c-e	2502	h	1132	h	2252	bc	882	b-d
	2	17.5	b	1.47	e-g	67.2	ab	4.3	b	2084	i	809	hi	2068	e-g	792	de
	3	16.0	c	1.99	d-g	68.8	a	4.6	b	2004	i	705	i	2082	d-g	783	de
	4	19.5	a	3.15	cd	68.4	ab	5.7	a	2001	i	719	i	1902	h	620	f
HPMC	1	7.0	e	1.59	e-g	54.0	c	3.0	e	6378	bc	5058	c	2252	bc	932	a-c
	2	6.9	e	1.85	e-g	66.3	ab	3.0	e	5975	c-e	4674	d-f	2277	bc	976	a-c
	3	6.8	e	2.53	c-e	66.7	ab	3.1	de	5640	e	4378	f	2211	cd	950	a-c
	4	8.5	e	4.43	ab	59.6	a-c	3.1	de	5618	e	4411	f	2194	cd	987	ab

[a] Mean of duplicates. Values followed by different letters in each column and each starch are significant different ($p \leq 0.05$).

[b] WHC, water holding capacity; [c] n.d.= not determined.

545 **Table 2**. Effect of hydrocolloids on enzymatic hydrolysis kinetics of corn and potato starches[a,b].
546

Blends	Level (%)	C∞		*k*		AUC 180		H90		HI		eGI	
Corn		87.6	cd	0.0155	f	1864	k	65	l	62	k	62	l
Pectin	1	89.0	c	0.0669	c-f	2510	c-e	88	cd	84	c-e	80	c-e
	2	85.7	de	0.0304	ef	2208	j	79	ij	74	j	72	j
	3	74.6	k	0.1372	b	2180	j	74	k	73	j	71	j
	4	78.2	j	0.1115	b-d	2328	i	78	j	78	i	75	i
Guar gum	1	86.0	de	0.1355	bc	2453	de	86	ef	84	de	80	de
	2	83.1	hi	0.2481	a	2456	ef	83	g	82	ef	79	ef
	3	80.9	hi	0.1241	b-d	2361	gh	80	hi	80	gh	77	gh
	4	87.7	cd	0.0852	b-e	2542	cd	87	c-e	85	cd	82	cd
CMC	1	92.1	b	0.1144	bc	2683	b	92	b	89	b	86	b
	2	98.2	a	0.0842	b-e	3134	a	97	a	105	a	98	a
	3	93.5	b	0.1166	b-d	2746	b	93	b	92	b	87	b
	4	92.6	b	0.1207	b-d	2713	b	92	b	91	b	87	b
Xanthan	1	81.7	g-i	0.1068	b-d	2384	gh	81	gh	80	gh	77	gh
	2	80.9	f-h	0.0954	b-e	2347	g-i	80	hi	78	g-i	76	g-i
	3	79.6	ij	0.0807	b-e	2359	hi	80	hi	79	hi	76	hi
	4	83.2	f-h	0.1147	b-d	2428	fg	83	g	81	fg	78	fg
HPMC	1	89.3	c	0.0842	b-e	2514	c	89	c	86	c	82	c
	2	85.3	ef	0.0949	b-e	2475	ef	85	f	82	ef	79	ef
	3	83.0	d-g	0.0652	e-f	2364	ef	83	g	79	g-i	77	g-i
	4	87.9	cd	0.0631	d-f	2497	de	87	de	83	de	80	de
Potato	0	78.4	jk	0.0123	fg	2043	f	51	hi	49	f	51	f
Pectin	1	85.0	d-g	0.1131	bc	3370	a	85	bc	81	a	80	a
	2	88.3	c-f	0.1436	ab	3646	a	88	ab	88	a	84	a
	3	92.0	bc	0.1553	a	3725	a	92	ab	90	a	86	a
	4	89.8	b-d	0.1202	ab	3714	a	90	a	89	a	86	a

Guar gum	1	76.0	jk	0.0622	de	2989	b	75	cd	73	b	70	b
	2	86.9	c-g	0.0065	g	1682	g	38	j	40	g	43	g
	3	89.1	b-d	0.0076	g	1855	g	45	j	44	g	46	g
	4	92.1	bc	0.0064	g	1669	g	41	j	40	g	44	g
CMC	1	84.2	f-i	0.0186	fg	2514	c-f	68	d-h	60	c-f	61	c-f
	2	84.2	f-i	0.0197	fg	2548	b-f	67	d-h	61	c-f	62	b-f
	3	77.6	i-k	0.0166	fg	2329	ef	62	g-i	55	ef	57	ef
	4	76.0	k	0.0468	ef	2787	b-d	76	de	68	bc	68	bc
Xanthan	1	97.0	ab	0.0382	e-g	3470	a	94	ab	84	a	82	a
	2	77.3	k	0.0202	fg	2347	d-f	63	f-j	57	d-f	58	d-f
	3	64.5	l	0.0835	cd	2527	c-f	64	f-i	61	c-f	60	c-f
	4	77.1	k	0.0213	fg	2454	c-f	65	e-i	59	c-f	59	c-f
HPMC	1	86.5	c-e	0.0197	fg	2863	b-d	75	d-f	66	b-d	66	b-d
	2	98.6	a	0.041	e-g	3679	a	97	a	89	a	86	a
	3	83.5	g-j	0.0242	fg	2647	b-e	71	d-g	64	b-f	64	b-e
	4	82.6	h-k	0.0226	fg	2548	c-f	68	d-h	62	b-f	62	b-f

547 a Mean of four replicates. Values followed by different letters in each column and each starch indicate significant differences ($p \leq 0.05$).
548 [b] $C\infty$, equilibrium concentration; *k*, kinetic constant; HI, hydrolysis index; AUC 180, area under curve; eGI, estimated glycemic index.
549