Carbohydrate Polymers 273 (2021) 118549

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Estimation of viscosity and hydrolysis kinetics of corn starch gels based on microstructural features using a simplified model

Maria Santamaria [a], Raquel Garzon [a], Ramón Moreira [b], Cristina M. Rosell [a,*]

[a] Institute of Agrochemistry and Food Technology (IATA-CSIC), C/Agustin Escardino, 7, 46980 Paterna, Spain
[b] Department of Chemical Engineering, Universidade de Santiago de Compostela, rúa Lope Gómez de Marzoa, Santiago de Compostela, E-15782, Spain

ARTICLE INFO

Keywords:
In vitro digestibility
Microstructure
Modelling
Starch content

ABSTRACT

Viscosity is an important rheological property, which may have impact on the glycemic response of starchy foods. However, the relationship between starch gels viscosity on its hydrolysis has not been elucidated. The aim of this work was to assess the effect of gels viscosity on the microstructure, and the kinetics of enzymatic hydrolysis of starch. Corn starch gels were prepared from starch:water ratios varying from 1:4 to 1:16. A structural model was proposed that correlated (R-square = 0.98) the porous structure (cavity sizes, thickness walls) of gels and its viscosity. Kinetics constants of hydrolysis decreased with increasing starch content and consequently with gel viscosity. Relationships of viscosity with the microstructural features of gels suggested that enzyme diffusion into the gel was hindered, with the subsequent impact on the hydrolysis kinetics. Therefore, starch digestibility could be governed by starch gels viscosity, which also affected their microstructure.

1. Introduction

The understanding of starch hydrolysis is attracting much research owing its relationship with the metabolic processes occurring along human digestion, particularly the postprandial blood glucose levels (Hardacre, Lentle, Yap, & Monro, 2016). Previous to the glucose absorption in small intestine, starch is hydrolyzed by salivary and pancreatic α-amylase in the mouth and small intestine, respectively, generating short oligomers, such as maltose or maltotriose (Dona, Pages, Gilbert, & Kuchel, 2010). According to the rate of hydrolysis, starch is commonly categorized into three fractions (Englyst & Hudson, 1996): rapidly digestible starch (RDS) associated with a fast increase in blood glucose level, slowly digestible starch (SDS) slowly hydrolyzed in the small intestine, and resistant starch (RS), which is not digested by the enzymes in the superior gastrointestinal tract, but microorganisms can ferment it to short chain fatty acids (SCFA) in the large intestine (Dura, Rose, & Rosell, 2017; Zhou et al., 2020).

Despite the interest in starch digestion, there is uncertainty about the factors that could affect the hydrolysis of starch catalyzed by α-amylase. The starch concentration, its botanical origin, or the starch status as native or gelatinized form are important properties that may influence the hydrolysis. Previous studies suggested that cereal flours are digested more rapidly than tubers and legume flours, due to their difference in starch microstructure and chemical composition (Gularte & Rosell, 2011; Liu, Donner, Yin, Huang, & Fan, 2006). Furthermore, Dhital, Warren, Butterworth, Ellis, and Gidley (2017) described that mechanisms limiting enzymatic activity are related to binding or blocking the access of α-amylase. Those authors differentiated when enzymatic hydrolysis is in aqueous solution as occurs in the gelatinized starch or in slurry as the case of granular starch. In both cases the amylase hydrolysis might be limited by, first the barriers that prevent the binding of the enzyme to starch and secondly, the structural features of starch that impede amylase access to the substrate. Consequently, physical characterization of the starch granule as size, pores in the granular surface or the supramolecular structure are properties that can impact the adsorption and binding of the α-amylase. Besides starch structure, viscosity of the system has been incorporated as one important element in the starch digestion (Hardacre, Lentle, Yap, & Monro, 2016). However, studies investigating viscosity have been focused on the impact of soluble and insoluble dietary fiber, but not on the role of gels viscosity produced as a result of starch gelatinization. The addition of hydrocolloids (usually labelled as non-starch polysaccharides, NPS) modifies the gelatinization/gelation process of the starch (Brennan, Suter, Luethi, Matia-Merino, & Qvortrup, 2008; Tomoko & Kaoru, 2011). A study

* Corresponding author at: Institute of Agrochemistry and Food Technology (IATA-CSIC), C/Agustin Escardino, 7, 46980 Paterna, Spain.
E-mail addresses: masanar@iata.csic.es (M. Santamaria), r.garzon@iata.csic.es (R. Garzon), ramon.moreira@usc.es (R. Moreira), crosell@iata.csic.es (C.M. Rosell).

https://doi.org/10.1016/j.carbpol.2021.118549
Received 15 April 2021; Received in revised form 6 August 2021; Accepted 8 August 2021
Available online 11 August 2021

carried out with corn and potato starches and different hydrocolloids (pectin, guar gum, xanthan gum and soluble cellulose derivatives CMC and HPMC) confirmed that hydrocolloids affected the hydrolysis rate to different extent, depending on the hydrocolloid and type of starch (Gularte & Rosell, 2011). Authors observed an increase in initial rate of starch amylolysis in the presence of hydrocolloids, with the exception of guar gum that decreased the kinetic constant in potato gels (Gularte & Rosell, 2011). Yuris, Goh, Hardacre, and Matia-Merino (2019) studied the digestibility of wheat starch gels in the presence of several polysaccharides (xanthan, guar, agar) and explained the reduction in the starch digestibility by the increase in gel hardness that limits the enzyme accessibility to starch. Similarly, guar and xanthan gums added to high-amylose corn starch affected starch viscosity and retarded starch hydrolysis leading to lower estimated glycemic response (Chung, Liu, & Lim, 2007; Zhang, Li, You, Fang, & Li, 2020). The different studies discussed the relationship between the extent of starch hydrolysis and the system viscosity, but divergences on the role of viscosity accelerating or slowing down the starch hydrolysis have been encountered, which might be attributed to a possible viscosity threshold required for that enzymatic inhibition. Additionally, some studies analyzed the relation between insoluble fiber like cellulose and the α-amylase activity. Nsor-atindana, Yu, Goff, Chen, and Zhong (2020) reported that amylase can bind cellulose and act as a reversible and non-specific inhibitor, and the inhibition becomes more apparent as the particle size of the polymer decreases (Dhital, Gidley, & Warren, 2015; Nsor-atindana, Yu, Goff, Chen, & Zhong, 2020).

Therefore, although it has been found out that the viscosity of exogenous sources of hydrocolloids impacts the rate of digestive hydrolysis of starch to our best knowledge there are no studies regarding the viscosity effect of starch gels on their hydrolysis by digestive enzymes. Based on this, we initially hypothesized that starch gels viscosity could affect their digestion, and furthermore, that their structural features also might influence the enzymes accessibility to the starch. The aim of this study was to unravel the impact of viscosity and gel microstructure on the enzymatic hydrolysis of starch gels, using homogeneous gels prepared only with starch, in order to avoid possible artifacts derived from the interaction between heterologous polymers as it occurs in the presence of different hydrocolloids. Corn starch gels were prepared with different starch concentrations leading to gels with different properties and microstructure. To simulate starch digestion, the oro-gastrointestinal digestion (Minekus et al., 2014) and a direct *in vitro* enzymatic hydrolysis (Benavent-Gil & Rosell, 2017) were applied to the different gels.

2. Materials and methods

2.1. Materials

Corn starch EPSA (Valencia, Spain) of 95% purity (20.25% amylose content) and 13.22% moisture content was used. The enzymes used were type VI-B α-amylase from porcine pancreas (EC 3.2.1.1), pepsin from porcine gastric mucosa (EC 3.4.23.1), pancreatin from porcine pancreas (EC 232.468.9), bile salts and 3,5-dinitrosalicylic acid (DNS) were acquired from Sigma Aldrich (Sigma Chemical, St. Louis, USA). Amyloglucosidase (EC 3.2.1.3) was provided by Novozymes (Bagsvaerd, Denmark). Glucose oxidase/peroxidase (GOPOD) kit (Megazyme International Ireland Ltd., Bray, Ireland) was used. Solutions and standards were prepared by using deionized water. All reagents were of analytical grade.

2.2. Preparation of gels and pasting properties

The preparation of starch gels and the pasting performance of each samples was determined by Rapid Visco Analyzer (RVA 4500; Perten Instruments, Hägersten, Sweden). Corn starch gels were prepared at different concentrations with deionized water (w:w, 1:4; 1:6; 1:8; 1:10; 1:12; 1:14; 1:16). Slurries were subjected to heating and cooling cycles consisting of: 50 °C for 1 min, heating from 50 to 95 °C in 3 min 42 s, holding at 95 °C for 2 min 30 s, then cooling down to 50 °C in 3 min 48 s and holding at 50 °C for 2 min. The pasting parameters evaluated included the peak viscosity (maximum viscosity during heating), breakdown (viscosity difference between peak viscosity and trough), and the pasting rate calculated as the slope of the apparent viscosity during heating until 95 °C. The apparent viscosity of the formed gels was measured at 37 °C with a vibrational viscometer VL7-100B-d15 (Hydramotion Ltd., Malton, UK). This apparatus measures viscosity at high shear rate where the strong shear-thinning behavior of samples is less relevant. Moisture of gels was determined in two steps using an infrared balance (KERN, Balingen, Germany). Three different batches for each gel were prepared.

2.3. Total starch

The amount of total starch of the gels was quantified using a commercial assay kit (Megazyme International Ireland Ltd., Bray, Ireland). Two replicates were measured for each sample.

2.4. Scanning Electron Microscopy (SEM)

Fresh gels were immersed in liquid nitrogen and then freeze-dried. The microstructure of the different freeze-dried gels was observed using scanning electron microscopy (S-4800, Hitachi, Ibaraki, Japan). Samples were examined at an accelerating voltage of 10 kV and 100× magnification. Micrographs (1.3 × 0.98 mm) were captured. The microstructure analysis was carried out using the ImageJ analysis program (ImageJ, National Institutes of Health, Bethesda, Maryland, USA) and NIS-Elements software (Nikon Instruments Inc., Tokyo, Japan). An auto local thresholding was applied using ImageJ software and measured the wall thickness, and then the measurement of gel cavities or holes was carried out with Nis-Elements software. Parameters assessed were number of cavities/mm^2, mean cavity area (μm^2), porosity (%) calculated as ratio of total area of cavities and total image area, and wall thickness (μm) as previously described by Garzon and Rosell (2021). Three images were used to calculate the average of previous parameters.

2.5. In vitro oro-gastrointestinal digestion

The oro-gastrointestinal digestion was carried out following the standardized static digestion method described by Minekus et al. (2014) and adapted by Aleixandre, Benavent-Gil, and Rosell (2019). Minor modifications included the use of five grams of gel prepared in the Rapid Visco Analyzer (RVA) and 27 U/mL of α-amylase solution. Aliquots were withdrawn along digestion. Specifically, at the end of oral and gastric digestion and during the three hours of intestinal digestion. Aliquots were immediately heated to 100 °C for 5 min to stop enzyme hydrolysis. Hydrolysis was quantified with 3,5-dinitrosalicylic acid (DNS) spectrophotometrically using an SPECTROstar Nano microplate reader (BMG LABTECH, Ortenberg, Germany) at 540 nm, using maltose as standard. Resistant starch was determined at the end of the digestion.

2.6. Hydrolysis kinetics and expected glycemic index

Hydrolysis kinetics of starch gels were determined following the method described by Benavent-Gil and Rosell (2017) with minor modifications. One gram of gel was suspended into 4 mL of 0.1 M sodium maleate buffer (pH 6.9) with porcine pancreatic α-amylase (0.9 U/mL) and incubated in a shaker incubator SKI 4 (ARGO Lab, Carpi, Italy) at 37 °C under constant stirring at 200 rpm during 3 h. Aliquots (100 μL) were taken during incubation and mixed with 100 μL ethanol (96%) to stop the enzymatic hydrolysis. Then, it was centrifuged for 5 min (10,000 ×*g*, 4 °C). The pellet was suspended in 100 μL of ethanol (50%)

and centrifuged as described before. Supernatants were pooled together and kept at 4 °C. Supernatant (100 μL) was diluted with 885 μL of 0.1 M sodium acetate buffer (pH 4.5) and incubated with 15 μL amyloglucosidase (214.5 U/mL) at 50 °C for 30 min in a shaking incubator, before quantifying glucose content.

The remnant starch after 24 h hydrolysis was solubilized with 2 mL of cold 1.7 M NaOH. The mixture was homogenized with Polytron Ultra-Turrax T18 (IKA-Werke GmbH and Co. KG, Staufen, Germany) for 5 min at 14,000 rpm in an ice bath. The homogenate was diluted with 8 mL 0.6 M sodium acetate pH 3.8 containing calcium chloride (5 mM) and incubated with 100 μL AMG (143 U/mL) at 50 °C for 30 min in a shaking water bath. Afterwards, the glucose content was measured using a glucose oxidase–peroxidase (GOPOD). The absorbance was measured at 510 nm. Starch was calculated as glucose (mg) × 0.9.

The hydrolysis results allowed to calculate the amount of starch fractions. Rapidly digestible starch (RDS) was the starch fraction hydrolyzed within 20 min of incubation, slowly digestible starch (SDS) was the fraction hydrolyzed within 20 and 120 min, total digestible starch (DS) the amount of hydrolyzed starch after 24 h of incubation and resistant starch (RS) was the starch fraction that remained unhydrolyzed after 24 h of incubation (Calle, Benavent-Gil, & Rosell, 2020). The *in vitro* digestion kinetics were calculated fitting experimental data to a first-order equation (Eq. 1) (Goñi, Garcia-Alonso, & Saura-Calixto, 1997):

$$C = C_{\infty}\left(1 - e^{-kt}\right) \quad (1)$$

where C was the percentage of starch hydrolyzed at t time, C_{∞} was the equilibrium concentration or maximum hydrolysis of starch gels, k was the kinetic constant and t was the time chosen. In addition, the time required to reach 50% of C_{∞} (t_{50}) was calculated. The hydrolysis index (HI) was obtained by dividing the area under hydrolysis curve (0–180 min) of the sample by the area of the sample more concentrated (1:4) over the same period. The expected glycemic index (*e*GI) was calculated with the proposed Eq. (2) (Granfeldt, Björck, Drews, & Tovar, 1992).

$$e\mathrm{GI} = 8.198 + 0.862\ \mathrm{HI} \quad (2)$$

2.7. Statistical analyses

Experimental data were statistically analyzed using an analysis of variance (ANOVA) and values were expressed as mean ± standard deviation, using Statgraphics Centurion XVII software (Statistical Graphics Corporation, Rockville, MD, USA). Fisher's least significant differences test (LSD) was used to estimate significant differences among experimental mean values. Differences of $P < 0.05$ were considered significant. Furthermore, Pearson correlation analysis was used to identify possible relationships among experimental parameters.

3. Results and discussion

3.1. Formation process of gel

The pasting properties were recorded to identify the impact of starch concentration on the gel performance. Rapid Visco Analyzer (RVA) registered the apparent viscosity during heating and cooling cycle; the logarithmic scale for the apparent viscosity was used for comparison purposes (Fig. 1). The pasting behavior in RVA cycle was different among samples. At high starch content the maximum peak viscosity was reached earlier with higher slope (pasting rate) during heating, indicating faster increase of apparent viscosity. Peak viscosity is considered the equilibrium point between swelling and rupture of starch granules (Balet, Guelpa, Fox, & Manley, 2019). Therefore, at low starch content the granules can swell more freely, without the contact of other swollen granules. In consequence the rupture was delayed and reached at higher temperatures. As a result, the peak temperature decreased from 95 to 84 °C with increasing starch content. Eerlingen, Jacobs, Block, and Delcour (1997) reported similar performance when different concentrations of potato starch were subjected to different hydrothermal treatments. At low concentrations, the starch particles are completely swollen, but the space is rather limited at a higher starch concentration and swollen granules can only fill up the available space referred as close packing concentration. At the lowest concentration, a shoulder was visible before reaching the maximum peak viscosity, likely evidencing differences in swelling rate of starch granules associated to their particle size distribution. It has been reported that the average size of individual corn starch granules ranged within 1–7 μm for small and 15–20 μm for large granules (Singh, Singh, Kaur, Singh Sodhi, & Singh Gill, 2003). Mishra and Rai (2006) observed that corn starch exhibited polyhedral granules with size ranging from 3.6 to 14.3 μm. Differences in the granular size led to diverse surface area that could interact with water, and in consequence modifying the swelling rate. Nevertheless, the viscosity shoulder was only visible in the more diluted system, probably at higher concentration the predominant granules size population masked the swelling of the less abundant one.

Regarding the maximum apparent viscosity, as expected, the most concentrated starch gel (starch:water, 1:4) showed the highest peak of

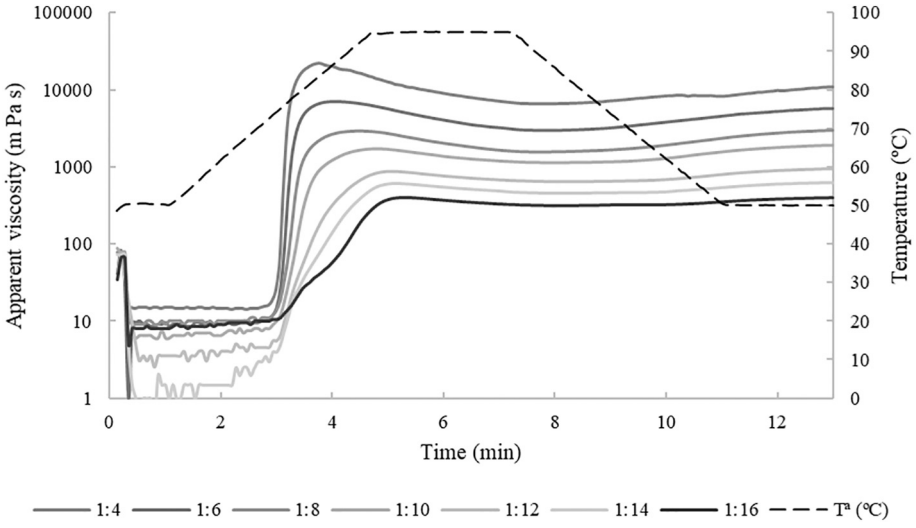

Fig. 1. RVA pasting profiles of corn starch gels prepared with different starch concentrations. Values in the legend are referred to the ratio starch:water (w:w). Discontinuous line shows the temperature applied during the heating-cooling cycle.

apparent viscosity (21,727 mPa s), observing a progressive decrease of that viscosity when increasing the starch dilution up to 1:16. Similar trend was observed in the final viscosity. This result was expected based on the amount of starch added in each slurry, because the apparent viscosity was directly related to the amount of starch.

The viscosity decay observed along holding at 95 °C (breakdown), associated with the disintegration degree of starch granules, exhibited also differences among samples. Major differences were observed within the most concentrated gels up to 1:8, at higher dilution changes in apparent viscosity were less visible, even during cooling. Standard methods for recording apparent viscosity of starches are usually carried out with starch:water slurries of 1:8, obtaining pasting profiles similar to the present study (Calle, Benavent-Gil, & Rosell, 2021; Mishra & Rai, 2006). Nevertheless, no previous study showed the apparent viscosity of gels with different starch concentration and how it impacts on the starch digestibility.

3.2. Characterization of the gels

Considering the potential impact of gels characteristics on their hydrolysis performance, a thorough analysis of the gels was carried out. Viscosity at 37 °C and the content of total starch in tested gels are presented in Table 1. The total starch content decreased as the dilution increased. The wide range of gels concentrations, from 4.5% to 18.6%, could cover the concentration existing in very diverse starch foods, from soups to salad dressings (4–15%). As expected, starch concentration had a significant impact on the gels' viscosity (R-square = 0.97). Sample with the highest content of total starch (18.6%) also showed the highest viscosity (768 mPa s). Conversely, the viscosity of the more diluted gel was 48 mPa s. A significant power law correlation was observed between the starch content and the resulting gels viscosities, which was related to the change on flow resistance when modifying the amount of solid per volume unit (Moreira, Chenlo, Torres, & Glazer, 2012).

The structural impact of starch concentration on the resulting gels was evaluated by analyzing the SEM micrographs (Fig. 2). The gels morphology considerably varied with the starch content. Gel microstructure resembled a network with small cavities. As the starch dilution increased, an enhancement in the size of cavities was observed with samples 1:4 and 1:6 having more closed structures (Fig. 2a and b). The disintegration of granules during heating, as indicated the breakdown observed for those gels in the RVA, might be responsible for that tight structure. The results of the image analysis (Table 1) confirmed significant differences ($P < 0.05$) in the microstructure of the gels, except for porosity. The number of cavities or holes in the gels showed a steady decrease as the starch dilution increased up to 1:8. Further dilutions did not induce significant differences in the number of cavities/mm^2. Simultaneously, the mean area of the cavities progressively increased with the starch dilution in the gels, again until sample 1:8, with no additional changes at higher dilution values. There was a significant positive relationship between number of cavities with viscosity (R-square = 0.87) and total starch (R-square = 0.82). Conversely, negative significant relationships were obtained between the mean area of the cavities with viscosity (R-square = −0.84) and total starch (R-square = −0.84). When the median area of the cavities was used for comparing gels, the same trend was observed, except for the gel with the highest dilution (1:16) that exhibited significantly larger cavities.

Possible relationships among starch content, gels microstructure and their viscosity were analyzed. There was a positive logarithmic relationship (R-square = 0.98) between the thickness of the cavities' walls and the starch content of the gels, and exponential with the gels' viscosity (R-square = 0.94). It was expected that the apparent viscosity of the gels depends mainly on the solid content, but viscosity values (Table 1) suggested that the 3-D network of the gel and its spatial distribution also must be considered. The gel structures shown in Fig. 2 were modelled as follows: pores (with an equivalent radius, r_{eq}) given by the median cavity area (A) and walls whose thickness (e) can be

Table 1
Characterization of corn gels: total starch, viscosity at 37 °C and microstructure parameters.

Sample	Total starch (g/100 g gel)	Viscosity (mPa s)	No. cavities/mm^2	Mean cavity area (μm^2)	Median cavity area (μm^2)	Porosity (%)	Wall thickness (μm)	W_{eq} [a]
1:4	18.6 ± 0.1^{a}	768 ± 23^{a}	226 ± 9^{a}	2591 ± 119^{b}	1027 ± 134^{d}	59.9 ± 3.7^{ab}	9.1 ± 1.1^{a}	24.9 ± 1.8^{a}
1:6	11.2 ± 0.2^{b}	422 ± 27^{b}	175 ± 60^{ab}	3221 ± 1432^{b}	1613 ± 946^{cd}	58.0 ± 4.7^{ab}	7.3 ± 0.2^{b}	14.8 ± 2.1^{b}
1:8	8.7 ± 0.2^{c}	112 ± 30^{c}	100 ± 16^{bc}	6259 ± 685^{a}	3321 ± 130^{bc}	61.6 ± 3.8^{ab}	5.8 ± 0.3^{cd}	6.5 ± 0.7^{c}
1:10	7.2 ± 0.1^{d}	111 ± 15^{c}	88 ± 22^{c}	7709 ± 2155^{a}	5493 ± 2371^{ab}	66.3 ± 2.2^{a}	4.8 ± 0.0^{de}	3.4 ± 1.3^{d}
1:12	5.7 ± 0.1^{e}	74 ± 1^{d}	93 ± 14^{c}	7650 ± 246^{a}	5209 ± 520^{b}	69.8 ± 8.5^{a}	3.5 ± 0.3^{ef}	2.7 ± 0.6^{de}
1:14	5.4 ± 0.1^{e}	62 ± 7^{de}	122 ± 4^{bc}	7050 ± 1750^{a}	4691 ± 117^{b}	65.5 ± 7.7^{ab}	2.7 ± 0.2^{fg}	2.4 ± 0.4^{de}
1:16	4.5 ± 0.0^{f}	48 ± 4^{e}	93 ± 33^{c}	8806 ± 930^{a}	7668 ± 871^{a}	65.8 ± 3.6^{ab}	1.8 ± 0.2^{g}	1.0 ± 0.3^{e}
P-value	0.0001	0.0001	0.0050	0.0012	0.0009	0.2623	0.0001	0.0001

Means within the same column followed by different letters indicate significant differences $P < 0.05$.
[a] W_{eq} was obtained from Eq. (4): $W_{eq} = A_{TP(1:16)}/A_{TP} \cdot e/e_{1:16}$.

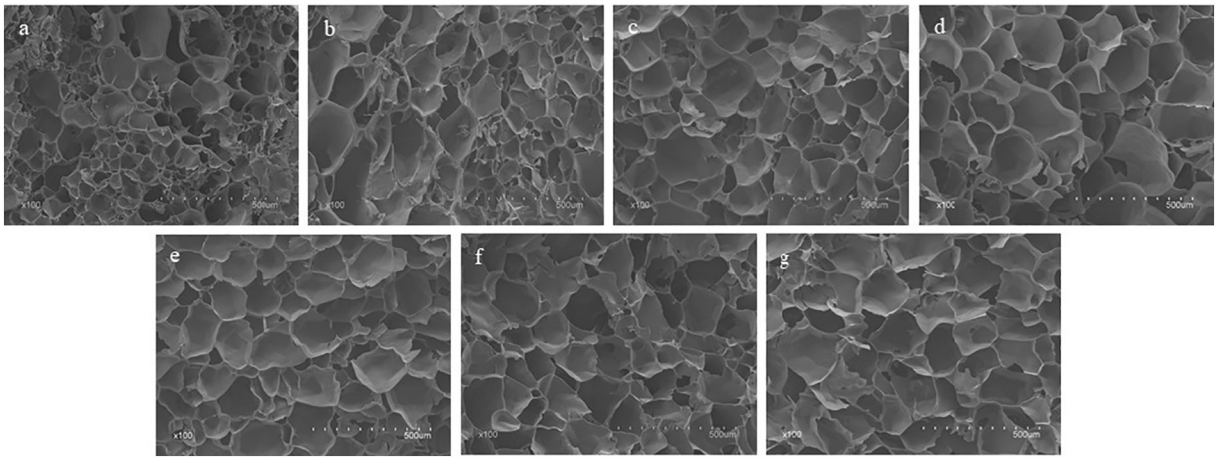

Fig. 2. Scanning electron micrograph of corn starch gels. Magnification 100×. The starch:water ratio is: 1:4 (a); 1:6 (b); 1:8 (c); 1:10 (d); 1:12 (e); 1:14 (f); 1:16 (g).

considered as two semi-thicknesses by the contribution of each neighboring pore covering. The area occupied by starch walls (A_{TP}) in relation to porous area can be evaluated by:

$$\frac{A_{TP}}{A} = \frac{A_e + A_s - A}{A} = \frac{A_e + A_s}{A} - 1 = \frac{(\pi + \sqrt{3} - \pi/2)\,(r_{eq} + e/2)^2}{A} - 1 \tag{3}$$

where A_e is the area of the circle with radius given by the sum of r_{eq} and e; A_s is the area between three tangent circles with area A_e.

Spatial distribution of the starch and the thickness of the wall depended on the starch gel content. As r_{eq} was in all cases longer than e, the highest A_{TP} (Eq. 3) was obtained with the highest cavity area (in this case 1:16). A_{TP} is employed to evaluate the number of cavities equivalent to contain the same amount of starch than in other gels. Nevertheless, these cavities have thicker walls and the number of equivalent walls, W_{eq}, regarded to the reference wall (thinnest wall, $e_{1:16}$) must be evaluated by means of:

$$W_{eq} = \frac{A_{TP(1:16)}}{A_{TP}}\,\frac{e}{e_{1:16}} \tag{4}$$

Eq. (4) allows the determination of the number of the walls with the same thickness (1.8 μm) per unit of starch gel. Introducing the corresponding data collected in Table 1 and by evaluation of Eq. (3), the number of walls increased with increasing starch content from 1 (1:16) up to 24.9 (1:4). A linear relationship (R-square = 0.98) between number of equivalent walls (W_{eq}) and viscosity (μ, mPa s) was found, Eq. (5), achieving a structural model that involves the porous characteristics of starchy gels and a physical property such as viscosity.

$$\mu = 30.46\, W_{eq} - 14.97 \tag{5}$$

3.3. *In vitro* digestion and hydrolysis of gels

The method INFOGEST was used to simulate the digestion of corn starch gels in the oro-gastrointestinal tract (Fig. 3). Experimental results are displayed as g of hydrolyzed starch per 100 g of gel, since the *in vitro* method is directly based on the amount of food ingested, in this case gels. Starch hydrolysis during oral and gastric phase presented very low hydrolysis considering the percentage of starch hydrolyzed. This was already reported by Iqbal, Wu, Kirk, and Chen (2021) because of a short residence time during oral phase and the inhibition of α-amylase at low pH in the gastric phase. In the intestinal phase, there was only an initial increase in the amount of hydrolyzed starch, but no further changes were observed along the intestinal digestion time. The oro-gastrointestinal digestion did not show a trend with the different starch gels, although the most concentrated gel (1:4) exhibited the lowest level of starch hydrolysis (1.5 g of hydrolyzed starch/100 g gel).

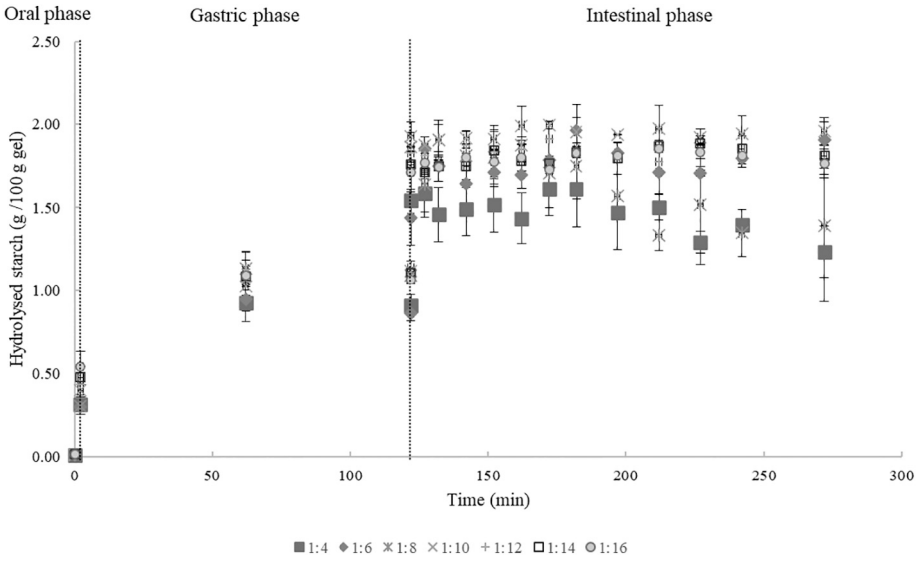

Fig. 3. *In vitro* oro-gastrointestinal digestion of gels prepared with different starch concentration. Legend is indicating the ratio starch:water used to prepare the gels.

Some authors indicated that samples with high starch content underwent slow hydrolysis, which has been related with the viscosity impeding the diffusion of enzymes, and in consequence, the enzymes accessibility and their binding to their substrate (Sanromán, Murado, & Lema, 1996; Wu et al., 2017).

Overall, the application of the oro-gastrointestinal *in vitro* digestion to starch gels did not allow us to identify the possible impact of gels viscosity and microstructure on the enzymatic hydrolysis, since the progressive dilution of the samples in each digestion phase masked differences associated to intrinsic characteristics of the gels. For this reason, the starch hydrolysis was directly carried out with porcine pancreatic α-amylase following methodology previously reported (Benavent-Gil & Rosell, 2017).

According to the rate and extent of *in vitro* digestion of starch, rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) were quantified, obtaining significant differences ($P < 0.05$) among the gels (Table 2). RDS, starch digested in the first 20 min, is the fraction that causes rapid increase in blood glucose after digestion of carbohydrates (Dona, Pages, Gilbert, & Kuchel, 2010). In this study, RDS did not present a linear correlation with the starch concentration. Sample 1:8 showed the highest amount of RDS. According to Dhital, Warren, Butterworth, Ellis, and Gidley (2017), the hydrolytic activity of the amylase could be reduced when the enzyme access to the starch is limited. In the present system, a decrease of the RDS might be expected when increasing gel viscosity, and thus the starch concentration of the gel. Nevertheless, that decrease was only observed at higher starch concentrations until 1:8, which suggests that a viscosity threshold was required in order to affect the enzyme accessibility. Conversely, SDS, related to low postprandial glycemic peak, showed steady decrease with the starch concentration, and the more diluted samples led to lower SDS. Chung, Liu, and Lim (2007) found that the incorporation of hydrocolloids increased the SDS, but without any clear trend on RDS content. Namely, samples with higher content of starch (1:4; 1:6) showed greater differences. Predictably, as the starch content in the gels was reduced, DS and RS decreased. Differences in DS were narrowed from sample 1:8 to 1:16, probably related to their viscosity differences at 37 °C (Table 1). Concerning RS, the amount of this fraction was directly related to the total starch amount of the gels.

For the more concentrated samples greater difference in viscosity was observed and the same trend was seen in the *in vitro* digestion parameters. Again, significant relationships were encountered with viscosity and the hydrolysis fractions SDS (R-square = 0.95) and RS (R-square = 0.96); and also the area of the cavities with SDS (R-square = −0.87) and RS (R-square = −0.84). The fraction of RDS content in relation to the initial starch content of the gel, *RDS*(%), decreased from 79.8% (1:16) up to 18.9% (1:4) with increasing starch content. It is worthy to mention that *RDS%* could be satisfactorily related with the structural parameter, W_{eq}, Eq. (4), by means of:

$$RDS\% = 74.45 - 16.73\ log\left(W_{eq}\right) \quad (6)$$

Table 2
Parameters of *in vitro* corn starch gels digestibility: rapidly digestible starch (RDS), slowly digestible starch (SDS), digestible starch (DS), resistant starch (RS).

Sample	RDS (g/100 g)	SDS (g/100 g)	DS (g/100 g)	RS (g/100 g)
1:4	3.51 ± 0.49^{bcd}	5.68 ± 1.16^{a}	9.99 ± 0.55^{a}	3.63 ± 0.24^{a}
1:6	3.77 ± 0.04^{ab}	3.64 ± 0.04^{b}	7.73 ± 0.17^{b}	2.41 ± 0.17^{b}
1:8	4.05 ± 0.22^{a}	1.95 ± 0.36^{c}	5.58 ± 0.69^{c}	1.59 ± 0.24^{c}
1:10	3.46 ± 0.18^{bcd}	1.57 ± 0.02^{c}	5.24 ± 0.67^{cd}	1.32 ± 0.13^{cd}
1:12	3.07 ± 0.07^{d}	1.43 ± 0.20^{cd}	4.17 ± 0.49^{de}	0.98 ± 0.06^{de}
1:14	3.14 ± 0.08^{cd}	0.86 ± 0.10^{cd}	4.23 ± 0.50^{de}	0.85 ± 0.15^{e}
1:16	3.59 ± 0.06^{abc}	0.27 ± 0.05^{d}	3.96 ± 0.14^{e}	0.70 ± 0.12^{e}
P-value	0.0110	0.0001	0.0001	0.0001

Values within the same column followed by different letters indicate significant differences $P < 0.05$.

This relationship (R-square = 0.95) indicates that the presence of a high number of equivalent walls of starch results in a decrease of the initial amount of starch that is accessible by enzymes.

Starch hydrolysis of gels prepared with different concentration of corn starch is presented in Fig. 4. Results have been plotted as both the amount of hydrolyzed starch per 100 g of gels *vs* time and the amount of hydrolyzed starch per 100 g of starch *vs* time. Those two different graphs for expressing results were chosen to understand the role of starch concentration in the gels. Hydrolysis plots confirmed the different behavior of the gels depending on the starch concentration. Fig. 4A showed the initial starch hydrolysis with minor differences in the rate of hydrolysis but the maximum hydrolysis reached was dependent on the gels dilution. A progressive reduction in the maximum hydrolyzed starch was observed when increasing gels dilution. Samples with higher dilution (1:12; 1:14; 1:16) had a rapid initial hydrolysis but reached a plateau after hydrolyzing low amount of starch (*ca.* 4%) (Fig. 4A). Regarding the starch content of the gels, when hydrolysis was followed recording the amount of hydrolyzed starch per starch amount on the gels (g starch/100 g of starch) (Fig. 4B) the pattern was completely different. There was a slower hydrolysis in the more concentrated gels and faster hydrolysis in the diluted ones, which also reached higher hydrolysis extension (up to 86%), compared to the 53% hydrolysis observed in the gel 1:4. Other studies (Tomoko & Kaoru, 2011), reported the impact of viscosity, provided by the addition of different gums, on the decrease of the starch hydrolysis. Likewise, Ma et al. (2019) reported that the incorporation of pectin increased the viscosity in the gut lumen and showed slower rate of starch hydrolysis. This could be attributed to the formation of a pectin layer around starch granules that limited the access of enzymes. Conversely, in the present study, a homogenous system comprising only starch has been used and results confirm the real impact of viscosity on the starch hydrolysis.

The starch hydrolysis in all the gels showed a very good fitting (R-square = 0.96) to a first order kinetics model. The kinetics parameters derived from hydrolysis of gels including kinetics constant (k), equilibrium concentration of hydrolyzed starch (C_∞), area under the hydrolysis curve after 180 min (AUC 180), hydrolysis index (HI) and estimated glycemic index (*e*GI) are summarized in Table 3. These parameters were significantly ($P < 0.05$) different depending on the gel concentration. The kinetics constant (k) increased with the starch dilution and the time to reach 50% of the hydrolysis (t_{50}) showed a progressive decrease with the dilution. Therefore, more concentrated gels exhibited slower hydrolysis over the digestion time. At constant enzyme concentration and temperature of reaction, an increase of enzymatic reaction rate would be expected when increasing the substrate concentration. However, in the present gels, there is an increase of reaction rate when diluting the starch and therefore, when decreasing the amount of starch in the gels, suggesting that the formation of enzyme-substrate complexes depended on the own structural gel features. High starch content hinders the enzyme diffusion into the gel and macroscopically this resistance associated to the mass transport can be related to gel viscosity (previously related to microstructural gel features with the proposed model). In fact, the hydrolysis kinetics constant depended inversely on the gel viscosity (Fig. 5). Two different trends could be determined, associated with high (>100 mPa s) and low (<100 mPa s) viscosities corresponding to high (>7 g starch/100 g gel) and low (<7 g starch/100 g gel) amount of starch in the gels. At low viscosity range, the kinetics constant value drops linearly (R-square = 0.98) with gel viscosity. This regression allows the empirical prediction of enzymatic kinetics constant value (k_1 = 0.22 min^{-1}) at very low starch amount present in the gel (very low substrate concentration and gel viscosity assumed equal to water viscosity at 37 °C, 0.692 mPa s) (Lide, 2005). This kinetics constant value could be interpreted like the kinetics constant in absence of mass transfer resistances within gel. In fact, the kinetics constant values collected in Table 3 must be considered like a global kinetics coefficient where enzymatic reaction constant value (k_1, min^{-1}) and mass transfer coefficient (k_m, min^{-1}) are involved and the simplified relationship,

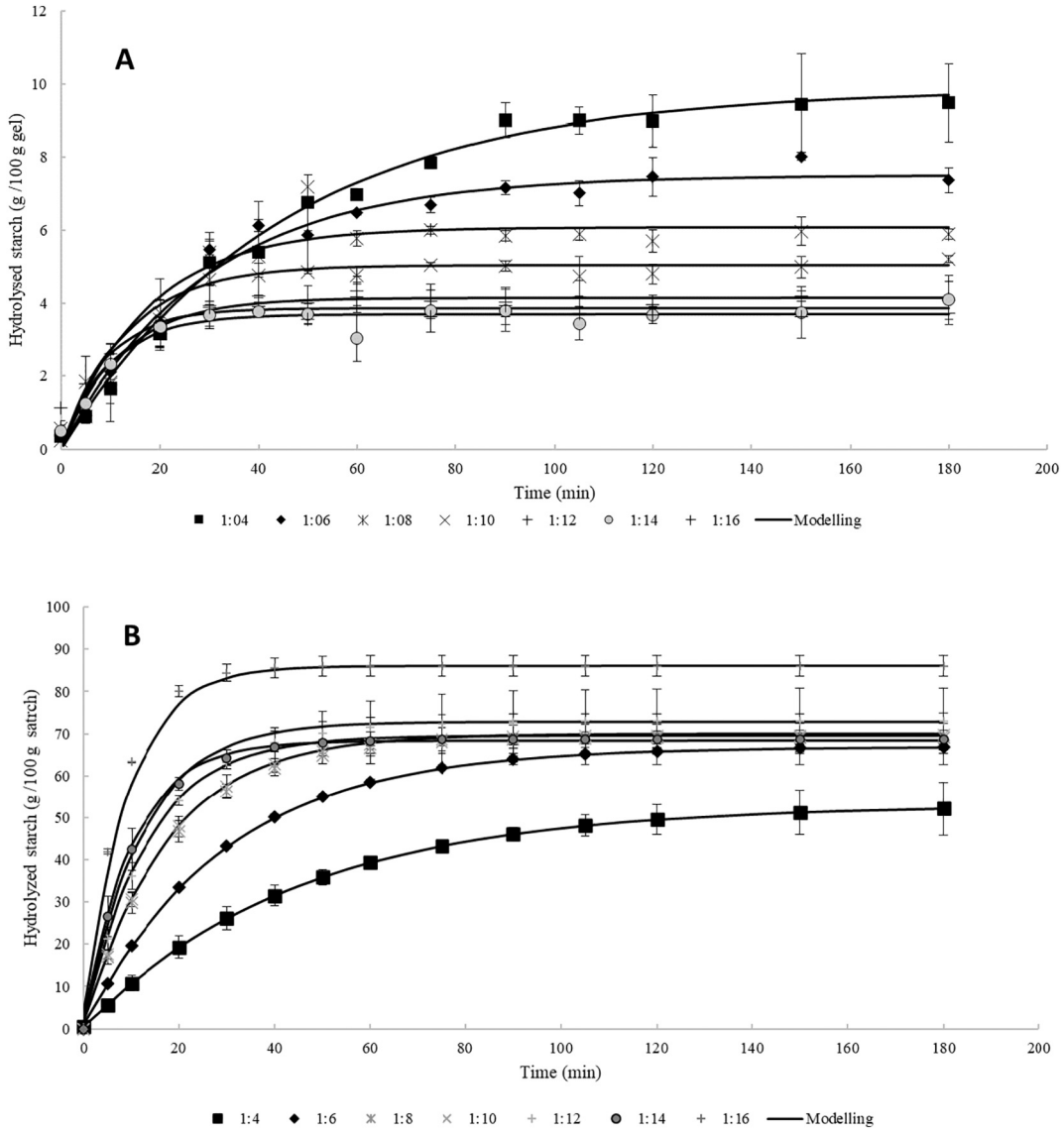

Fig. 4. Enzymatic starch hydrolysis of different corn starch gels prepared with different starch concentration. Legend is indicating the ratio starch:water used to prepare the gels. Hydrolysis plots are expressed as: g/100 g gel (A) and g/100 g starch (B). Solid lines correspond to first-order model with kinetics constant evaluated by Eq. (8).

Table 3
Kinetic parameters resulting from the enzymatic hydrolysis of corn gels with different starch concentrations. Kinetic parameters include: kinetic constant (k), time required to reach 50% of C_∞ (t_{50}); equilibrium concentration (C_∞), area under the hydrolysis curve after 180 min (AUC), hydrolysis index (HI) and estimated glycemic index (eGI) for corn gels with different concentration. Expressed per 100 g of gels (Fig. 4A).

Sample	k (min^{-1})	t_{50} (min)	C_∞ [a]	AUC	HI	*e*GI [b]	k_m [c] (min^{-1})
1:4	0.02 ± 0.01^{e}	35 ± 7^{a}	10.10 ± 1.53^{a}	1335.00 ± 49.50^{a}	100.00 ± 2.99^{a}	94.40 ± 2.58^{b}	0.02 ± 0.01^{e}
1:6	0.03 ± 0.00^{de}	20 ± 0^{b}	7.52 ± 0.08^{b}	1136.00 ± 12.73^{b}	85.09 ± 0.77^{b}	81.55 ± 0.66^{c}	0.04 ± 0.01^{de}
1:8	0.06 ± 0.01^{cd}	10 ± 0^{c}	6.01 ± 014^{c}	971.75 ± 8.27^{c}	72.79 ± 0.50^{c}	70.94 ± 0.43^{d}	0.07 ± 0.02^{cd}
1:10	0.06 ± 0.00^{cd}	10 ± 0^{c}	5.03 ± 0.20^{cd}	818.05 ± 34.29^{d}	61.28 ± 2.07^{d}	61.02 ± 1.79^{e}	0.08 ± 0.02^{cd}
1:12	0.07 ± 0.02^{bc}	10 ± 0^{c}	4.14 ± 0.44^{d}	683.65 ± 52.68^{e}	51.21 ± 3.18^{e}	52.34 ± 2.74^{f}	0.10 ± 0.02^{c}
1:14	0.10 ± 0.03^{ab}	8 ± 4^{c}	3.72 ± 0.33^{d}	628.00 ± 42.00^{e}	47.04 ± 2.54^{e}	48.75 ± 2.19^{f}	0.18 ± 0.03^{b}
1:16	0.13 ± 0.01^{a}	5 ± 0^{c}	3.86 ± 0.11^{d}	663.45 ± 17.04^{e}	49.70 ± 1.03^{e}	51.04 ± 0.89^{f}	0.34 ± 0.04^{a}
P-value	0.0004	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Values followed by different letters within a column denote significant differences ($P < 0.05$).
[a] C_∞ and k were determined by the equation, $C = C_\infty (1 - e^{-kt})$.
[b] *e*GI was quantified following the equation proposed by Granfeldt, Björck, Drews, and Tovar (1992).
[c] Obtained from Eq. (7): $1/k = 1/k_1 + 1/k_m$.

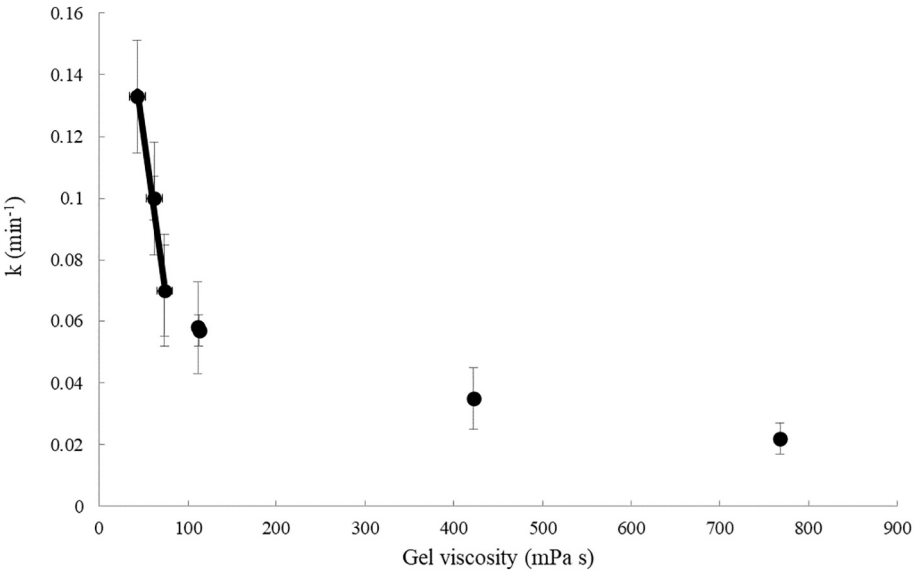

Fig. 5. Relationship of the kinetics constant of first order model with gel viscosity.

after several assumptions for a model of resistances in series, is given by the Eq. (7) (Levenspiel, 1998):

$$\frac{1}{k} = \frac{1}{k_1} + \frac{1}{k_m} \tag{7}$$

Eq. (7) allows the estimation of k_m of enzyme into the gels with different starch content and the corresponding values are shown in Table 3. The mass transfer coefficients value strictly depends on the characteristics of compound diffusing, turbulence conditions on the surface and properties of the fluid. In our case, in a simplified way, it was found a power relationship between k_m and viscosity (R-square = 0.996) and Eq. (7) can be written after substitution:

$$\frac{1}{k} = \frac{1}{0.22} + 0.196\,\eta^{0.8} \tag{8}$$

A very high correlation (R-square > 0.94) was obtained between experimental kinetics constant data and estimated values employing Eq. (8). The goodness of the first order model with the kinetics constant evaluated by Eq. (8) can be observed in the Fig. 4A and B. These results confirmed that the viscosity of starch gels must be considered to evaluate the hydrolysis rates. Previous hydrolysis studies dealing with changes in viscosity have been carried out with diverse hydrocolloids, and the slowdown of the enzymatic activity has been explained based on the hydrocolloid coating of the starch surface that block the enzyme accessibility to the substrate (Chung, Liu, & Lim, 2007; Gularte & Rosell, 2011). However, the present research confirmed the role of the apparent viscosity of the gels on the enzymatic hydrolysis.

In addition, the maximum hydrolysis (C_∞) reached with the different gels (Fig. 4A, Table 3) showed a significant decrease when increasing gels dilution. A similar trend was observed for the total area under the hydrolysis curve (AUC), which is related to the glucose released over a hydrolysis period of 180 min (Goñi, Garcia-Alonso, & Saura-Calixto, 1997). To estimate the glycemic index (*e*GI), the hydrolysis index (HI) of each gel was calculated taking the sample 1:4 as a reference (HI = 100%). The *e*GI showed a steady decrease until 51% in the most diluted sample. Glycemic index is used to describe how the food starch is hydrolyzed in the digestive system and absorbed into the bloodstream (Dona, Pages, Gilbert, & Kuchel, 2010). Some authors reported that the high viscosity induced by hydrocolloids might form a physical barrier for the α-amylase access, which would explain the decrease in glucose released and its absorption in the intestine (Dartois, Singh, Kaur, & Singh, 2010; Gularte & Rosell, 2011). Here, the same behavior was observed regarding the reduction in the hydrolysis rate, but now it is related to the increase of viscosity by the increase of starch content in the gels.

4. Conclusions

This study investigated for the first time the role of the viscosity of starch gels on the digestion of starch. Corn starch gels of varying starch concentration resulted in a range of different viscosities and microstructures. A structural model is proposed that connects by a linear relationship (R-square = 0.98) the porous structure (cavity sizes and thickness walls) of starch gels and their viscosity. The viscosity showed a linear relationship with the number of starch walls per area and its thickness (equivalent walls). The kinetics constant values of the starch hydrolysis decreased when increasing gel viscosity. Hydrolysis constants, considering mass transfer resistance within the gel, were successfully correlated with gel viscosity by means of a simple model, confirming the initial formulated hypothesis. Overall, the proposed simplified model links macrostructural properties (viscosity) and microstructural features (median cavity area and wall thickness) to analyze hydrolysis kinetics. It could also be extended to other physical and chemical processes where starch gels are involved and validated with other gels formed with starches from other sources. From the technological point of view, these findings could be applied in the design of food formulations aiming at postprandial glucose management.

Funding

Authors acknowledge the financial support of the Spanish Ministry of Science and Innovation (Project RTI2018-095919-B-C2) and the European Regional Development Fund (FEDER), Generalitat Valenciana (Project Prometeo 2017/189) and Xunta de Galicia (Consolidation Project ED431B 2019/01).

CRediT authorship contribution statement

Maria Santamaria: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Raquel Garzon:** Methodology, Supervision, Data curation. **Ramón Moreira:** Formal analysis, Writing – review & editing, Funding acquisition. **Cristina M. Rosell:** Conceptualization, Funding acquisition, Investigation, Supervision, Writing – review & editing.

Declaration of competing interest

None.

References

Aleixandre, A., Benavent-Gil, Y., & Rosell, C. M. (2019). Effect of bread structure and *in vitro* oral processing methods in bolus disintegration and glycemic index. *Nutrients, 11*(9), 2105.

Balet, S., Guelpa, A., Fox, G., & Manley, M. (2019). Rapid Visco Analyser (RVA) as a tool for measuring starch-related physiochemical properties in cereals: A review. *Food Analytical Methods, 12*(10), 2344–2360.

Benavent-Gil, Y., & Rosell, C. M. (2017). Performance of granular starch with controlled pore size during hydrolysis with digestive enzymes. *Plant Foods for Human Nutrition, 72*(4), 353–359.

Brennan, C. S., Suter, M., Luethi, T., Matia-Merino, L., & Qvortrup, J. (2008). The relationship between wheat flour and starch pasting properties and starch hydrolysis: Effect of non-starch polysaccharides in a starch gel system. *Starch - Stärke, 60*(1), 23–33.

Calle, J., Benavent-Gil, Y., & Rosell, C. M. (2020). Development of gluten free breads from *Colocasia esculenta* flour blended with hydrocolloids and enzymes. *Food Hydrocolloids, 98*, Article 105243.

Calle, J., Benavent-Gil, Y., & Rosell, C. M. (2021). Use of flour from cormels of *Xanthosoma sagittifolium* (L.) Schott and *Colocasia esculenta* (L.) Schott to develop pastes foods: Physico-chemical, functional and nutritional characterization. *Food Chemistry, 344*, Article 128666.

Chung, H. J., Liu, Q., & Lim, S. T. (2007). Texture and in vitro digestibility of white rice cooked with hydrocolloids. *Cereal Chemistry Journal, 84*(3), 246–249.

Dartois, A., Singh, J., Kaur, L., & Singh, H. (2010). Influence of guar gum on the in vitro starch digestibility—Rheological and microstructural characteristics. *Food Biophysics, 5*(3), 149–160.

Dhital, S., Gidley, M. J., & Warren, F. J. (2015). Inhibition of α-amylase activity by cellulose: Kinetic analysis and nutritional implications. *Carbohydrate Polymers, 123*, 305–312.

Dhital, S., Warren, F. J., Butterworth, P. J., Ellis, P. R., & Gidley, M. J. (2017). Mechanisms of starch digestion by α-amylase—Structural basis for kinetic properties. *Critical Reviews in Food Science and Nutrition, 57*(5), 875–892.

Dona, A. C., Pages, G., Gilbert, R. G., & Kuchel, P. W. (2010). Digestion of starch: *In vivo* and *in vitro* kinetic models used to characterise oligosaccharide or glucose release. *Carbohydrate Polymers, 80*(3), 599–617.

Dura, A., Rose, D. J., & Rosell, C. M. (2017). Enzymatic modification of corn starch influences human fecal fermentation profiles. *Journal of Agricultural and Food Chemistry, 65*(23), 4651–4657.

Eerlingen, R. C., Jacobs, H., Block, K., & Delcour, J. A. (1997). Effects of hydrothermal treatments on the rheological properties of potato starch. *Carbohydrate Research, 297*(4), 347–356.

Englyst, H. N., & Hudson, G. J. (1996). The classification and measurement of dietary carbohydrates. *Food Chemistry, 57*(1), 15–21.

Garzon, R., & Rosell, C. M. (2021). Rapid assessment of starch pasting using a rapid force analyzer. *Cereal Chemistry, 98*(2), 305–314.

Goñi, I., Garcia-Alonso, A., & Saura-Calixto, F. (1997). A starch hydrolysis procedure to estimate glycemic index. *Nutrition Research, 17*(3), 427–437.

Granfeldt, Y., Björck, I., Drews, A., & Tovar, J. (1992). An *in vitro* prodecure based on chewing to predict metabolic reponse to starch in cereal and legume products. *European Journal Clinical Nutrition, 46*, 649–660.

Gularte, M. A., & Rosell, C. M. (2011). Physicochemical properties and enzymatic hydrolysis of different starches in the presence of hydrocolloids. *Carbohydrate Polymers, 85*(1), 237–244.

Hardacre, A. K., Lentle, R. G., Yap, S. Y., & Monro, J. A. (2016). Does viscosity or structure govern the rate at which starch granules are digested? *Carbohydrate Polymers, 136*, 667–675.

Iqbal, S., Wu, P., Kirk, T. V., & Chen, X. D. (2021). Amylose content modulates maize starch hydrolysis, rheology, and microstructure during simulated gastrointestinal digestion. *Food Hydrocolloids, 110*, Article 106171.

Levenspiel, O. (1998). *Chemical reaction engineering* (3rd ed.). New York: Wiley.

Lide, D. R. (2005). *CRC handbook of chemistry and physics*. Boca Raton, FL: CRC.

Liu, Q., Donner, E., Yin, Y., Huang, R. L., & Fan, M. Z. (2006). The physicochemical properties and in vitro digestibility of selected cereals, tubers and legumes grown in China. *Food Chemistry, 99*(3), 470–477.

Ma, Y. S., Pan, Y., Xie, Q. T., Li, X. M., Zhang, B., & Chen, H. Q. (2019). Evaluation studies on effects of pectin with different concentrations on the pasting, rheological and digestibility properties of corn starch. *Food Chemistry, 274*, 319–323.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., … Brodkorb, A. (2014). A standardised staticin vitrodigestion method suitable for food — An international consensus. *Food & Function, 5*(6), 1113–1124.

Mishra, S., & Rai, T. (2006). Morphology and functional properties of corn, potato and tapioca starches. *Food Hydrocolloids, 20*(5), 557–566.

Moreira, R., Chenlo, F., Torres, M. D., & Glazer, J. (2012). Rheological properties of gelatinized chestnut starch dispersions: Effect of concentration and temperature. *Journal of Food Engineering, 112*(1–2), 94–99.

Nsor-atindana, J., Yu, M. H., Goff, H. D., Chen, M. S., & Zhong, F. (2020). Analysis of kinetic parameters and mechanisms of nanocrystalline cellulose inhibition of alpha-amylase and alpha-glucosidase in simulated digestion of starch. *Food & Function, 11*(5), 4719–4731.

Sanromán, A., Murado, M. A., & Lema, J. M. (1996). The influence of substrate structure on the kinetics of the hydrolysis of starch by glucoamylase. *Applied Biochemistry and Biotechnology, 59*(3), 329–336.

Singh, N., Singh, J., Kaur, L., Singh Sodhi, N., & Singh Gill, B. (2003). Morphological, thermal and rheological properties of starches from different botanical sources. *Food Chemistry, 81*(2), 219–231.

Tomoko, S., & Kaoru, K. (2011). Effect of non-starch polysaccharides on the *in vitro* digestibility and rheological properties of rice starch gel. *Food Chemistry, 127*(2), 541–546.

Wu, P., Bhattarai, R. R., Dhital, S., Deng, R., Chen, X. D., & Gidley, M. J. (2017). *In vitro* digestion of pectin- and mango-enriched diets using a dynamic rat stomach-duodenum model. *Journal of Food Engineering, 202*, 65–78.

Yuris, A., Goh, K. K. T., Hardacre, A. K., & Matia-Merino, L. (2019). The effect of gel structure on the *in vitro* digestibility of wheat starch-Mesona chinensis polysaccharide gels. *Food & Function, 10*(1), 250–258.

Zhang, Y., Li, M., You, X., Fang, F., & Li, B. (2020). Impacts of guar and xanthan gums on pasting and gel properties of high-amylose corn starches. *International Journal of Biological Macromolecules, 146*, 1060–1068.

Zhou, S., Hong, Y., Gu, Z., Cheng, L., Li, Z., & Li, C. (2020). Effect of heat-moisture treatment on the *in vitro* digestibility and physicochemical properties of starch-hydrocolloid complexes. *Food Hydrocolloids, 104*, 105736.