



Effect of the addition of different sodium alginates on viscoelastic, structural features and hydrolysis kinetics of corn starch gels

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ABSTRACT

Corn starch gels (1:4 w:w) were made with the addition (up to 2% w/w starch basis) of sodium alginates of different average molecular sizes and nature. Rheology of the starchy gels were characterized to evaluate their viscoelasticity. Gels were subjected to *in vitro* digestion to estimate the effect of alginates addition on glyceemic response. Tested alginates significantly decreased the value of the elastic modulus of gels. The coordination number obtained from complex modulus indicated that in general, less compact structures were obtained in the presence of alginates. These results were confirmed by scanning electron micrographs, where significant differences in the structure of alginate-corn starch gels were observed, but molecular size of alginates was not critical. Hydrolysis rate decreased with the presence of alginates in corn starch gels by interactions of alginates with leached amylose. At high alginate content, slower hydrolysis rates were determined when the most hydroscopic alginates were added.

1. Introduction

Sodium alginate (SA) is a polyuronic saccharide that is isolated from different brown seaweeds species around the world. SA is a copolymer composed of β -D-mannuronic acid (M) and α -L-guluronic acid (G). Alginates have extense industrial uses, like as thickener, in the paper industry, dye in textile industry, and binder in food industry (Kour et al., 2022). Copolymer features (M/G ratio, average block length, etc) depend on natural source, and molecular size is influenced by the process conditions employed during extraction, drying, and isolation, among other operations where alginate depolymerization could be promoted (Montes et al., 2021).

Starch is one of the most important functional food biopolymers. It can be extracted from many natural sources such as cereals, tubers and pulses. Starch is composed of two different glucose polymers: amylose, linear polymer, and amylopectin, branched polymer, joined by glycosidic bounds (Schwartz et al., 2014). Many hydrocolloids have been added to starchy systems to modify some features (texture, control moisture or water mobility) and improve the quality and stability of food products (Rosell et al., 2011). Sodium alginate (Feng et al., 2019), guar gum (Yadav et al., 2018), pectin (Fang et al., 2020), high

methoxylated pectin, guar gum, CMC, xanthan gum or HPMC (Gularte & Rosell, 2011) are among the hydrocolloids used in food applications. Particularly, alginate can be used to retard the starch retrogradation during cooling and storage stage. The interaction between alginate and starch displayed a retarding effect on the starch retrogradation, which was correlated with the amylose reaggregation and amylopectin recrystallization (Yu et al., 2018).

Regarding rheological effect of SA addition on starchy systems, several studies were reported. Li et al. (2017) found that the SA addition (0–4% w/w, starch basis) decreased the gel firmness, retrogradation enthalpy, storage modulus (G') and swelling power of corn starch gels obtained after heating at 95 °C for 10 min. Sun et al. (2013) showed that corn starch (10% w/w) with sodium alginate (1% w/w, solids total basis) decreased pasting temperature, peak and final viscosities, break down and setback values. Also, Ji et al. (2017) reported that corn starch (6% w/w) with sodium alginate (1% w/w, starch basis) decreased G' , complex viscosity and damping factor ($\tan \delta$) values. Same behaviour was reported for other starch sources. In fact, Feng et al. (2019) demonstrated that the addition (0.5% w/w, starch basis) of SA to sweet potato starch (10% w/w) decreased viscoelasticity and starch digestibility. However, Yang et al. (2021) studied rice starch (5% w/w

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water) added with SA (<0.50% w/v, starch basis) and observed a viscoelasticity increase. On the other hand, He et al. (2019) and Fang et al. (2020) showed that rice starch (10% w/w) and waxy potato starch (10% w/w), respectively, in presence of alginate (0.15% w/v and 1% w/w, starch basis) decreased G' and increased $\tan \delta$. These results showed that different effect of SA addition on rheological behaviour can be found in the literature and more research is necessary. Obviously, the experimental procedure to gelatinize the starch could partly explain the mentioned differences, but the somewhat the impact of SA characteristics can not be discarded.

The process of digestion involves the breakdown of a complex molecule (i.e., starch) into the simplest form (i.e., glucose). For simulating digestion processes, *in vitro* methods are widely used to study the intestinal behaviour of food (Sensoy, 2021). Most of the studies on the *in vitro* digestion of starch have focused on the intestinal stage. Using *in vitro* protocols, levels of rapidly digestible starch (RDS), slowly digestible starch (SDS), resistant starch (RS) and total starch (TS) of a gel can be calculated (Tuño et al., 2021). However, the effect of the gel structure on the starch digestion is poorly studied. Food structure can influence in the starch digestion (Zheng et al., 2021), and this fact is also valid for starch gels (Santamaria et al., 2021).

The incorporation of hydrocolloids in the food matrix could decrease the starch digestion due to blocking the interactions between substrate-enzyme or the direct impact on the active site of enzymes (Yemenicioğlu et al., 2020). Hydrocolloid's addition in starch-based food was associated with a decrease in starch digestion and related to a lower glycaemic index (Gularte & Rosell, 2011; Yuris et al., 2019). Furthermore, this impact was also reported with the addition of SA (Feng et al., 2019). This biopolymer could protect starch granules against normal swelling and form a barrier between enzyme-starch reducing the enzymatic activity. Ramírez et al. (2015) associated the effect of viscosity increase and the molecular interactions between alginate-starch with the lower glucose content during *in vitro* digestion. Nevertheless, previous studies on alginate effect on starch digestibility have not focussed on the specific characteristics of alginates and commercial ones have been used.

In this study, several sodium alginates with different average viscosimetric molecular weights isolated in lab from *Ascophyllum nodosum* brown seaweeds and commercial alginates were added to corn starch to determine the effects on rheological features during gel formation, structural features of formed gels and, finally, how the digestion kinetics are modified. Firstly, rheological properties and structure were analyzed. Secondly, the digestion kinetics were studied. Finally, relationships between starch gel structure and the digestion were established.

2. Materials and methods

2.1. Materials

Corn starch (CS) (initial moisture content of 12.25 g water · (100 g starch)⁻¹ (dry basis, d.b.)) was supplied by EPSA (Valencia, Spain). Commercial and lab-processed alginates were employed. Processed alginates from *Ascophyllum nodosum* seaweeds were obtained using a previously reported methodology (Montes et al., 2021), but drying temperature of seaweeds pellet was modified as follows: not dried (ND), dried at 50 °C (50D) and dried at 90 °C (90D). Furthermore, commercial sodium alginates (CAS No. 9005-38-3) from Sigma-Aldrich Chemical Company (S) (Lot MKCJ1280, St. Louis, MO, USA) and PanReac (P) (Lot 0F009964, Barcelona, Spain) were used as control (without knowledge of seaweeds source nor processing conditions). Average viscosimetric molecular weights (M_v , kg/mol), P (459 ± 8), S (156 ± 2), ND (428 ± 7), 50D (257 ± 1) and 90D (133 ± 1) of tested alginates were previously determined together with the corresponding average block length, mannuronate-guluronate ratio (Montes et al., 2021). The enzymes used were type VI-B α -amylase from porcine pancreas from Sigma-Aldrich Chemical Company (St. Louis, MO, USA) and amyloglucosidase from

Novozymes (Bagsvaerd, Denmark). D-Glucose Assay Kit (GOPOD) was purchased to Megazyme (Megazyme International Ireland Ltd., Bray, Ireland). All solutions were prepared using distilled water and standards were prepared using deionized water.

2.2. Preparation of starch gels

Alginates were dispersed in distilled water and hydrated overnight. Then, corn starch was weighed and added to the alginate solutions. These mixtures were manually shaken for 60 s to facilitate their homogenization. Then mixtures were heated at 100 °C for 20 min with gentle manual stirring every 5 min during 10 s before gel formation. The starch gels were cooled (for 20 min) up to 37 °C for later analysis. The gels were prepared at constant corn starch:water ratio (1:4), with different added alginate content (0, 1 and 2% w/w, starch basis).

2.3. Water retention capacity

Corn starch and alginate-corn starch mixtures were characterized by means of water retention capacity (WRC). WRC was determined following the protocol established by Kassem et al. (2022). Briefly, the samples (1.5 g) were weighed and directly added to centrifuge tubes containing 15 mL of distilled water or alginate solutions (previously hydrated overnight). After mixtures equilibration (18 h), samples were centrifuged at 3000 × g (7000 rpm in a 11192 rotor, Model 2–15, Sigma, Germany) at room temperature (22–25 °C) for 20 min. The supernatant was decanted, and fresh residue was weighed. Then, samples were dried up to constant weight (dry residue) at 70 °C under vacuum (<10 kPa). The WRC (kg kg⁻¹) was calculated by Equation (1).

$$WRC = \frac{\text{Fresh residue weight} - \text{Dry residue weight}}{\text{Dry residue weight}} \quad (1)$$

WRC of alginate-corn starch mixtures was employed to evaluate the WRC of alginates, WRC_{SA}, by means of the Equation (2):

$$WRC_{SA} = \frac{WRC_{mixture} - WRC_{CS} \left(1 - \frac{w_{SA}}{w_{dCS}}\right)}{\frac{w_{SA}}{w_{dCS}}} \quad (2)$$

where WRC_{CS} is the water retention capacity of corn starch, w_{dSA} and w_{dCS} are the dry weights of sodium alginate and starch in the mixture, respectively.

2.4. Rheological behaviour

The rheological characterisation was performed with a stress-controlled rheometer (MCR 301; Anton Paar Physica, Graz, Austria) using a starch pasting cell (ST24-2D/2V/2V-30) (gap = 2.460 mm, bob radius = 12.000 mm) with a solvent trap kit to minimize water evaporation during tests. The selected protocol was defined to simulate the experimental conditions which were employed during gel preparation (described in section 2.2). Corn starch and corn alginate-starch mixtures were dispersed in distilled water and introduced into the rheometer cuvette at 95 °C. Firstly, a pre-shear of 100 s⁻¹ was made for 1 min to homogenize the sample at 95 °C. Secondly, a time sweep was carried out at 95 °C for 19 min and then, a cooling ramp was performed from 95 to 37 °C at 3 °C/min at 30 Pa and 1 Hz in both stages. Then, strain sweep tests (0.1–10%) of gels at 1 Hz and 37 °C to obtain the linear viscoelasticity range (LVR) were performed (random strain sweep tests at 95 °C confirmed the validity of LVR measured at 37 °C in the range of tested temperatures). Finally, a second time sweep (30 Pa, 1 Hz) was carried out at 37 °C for 30 min to observe the maturation of the gel and a frequency sweep was made from 0.1 to 10 Hz at 1% strain and 37 °C. Complex modulus, G^* (Pa), values evaluated from G' and G'' data obtained through angular frequency (ω , Hz) sweep tests, were correlated to the following power law (Bruno et al., 2021), Equation (3):

$$G^* = \sqrt{(G')^2 + (G'')^2} = A w^{1/z} \quad (3)$$

where z (–) is the coordination number and measures number of rheological units with one another in the 3-D structure and A (G^* at 1 Hz) is called the proportional coefficient and evaluates the strength of the interaction between units.

2.5. Scanning electron microscopy (SEM)

Alginate-corn starch gels were freeze-dried and observed using the scanning electron microscopy (S-4800, Hitachi, Ibaraki, Japan). Gels were covered with gold by vacuum evaporator (JEE 400; JEOL, Tokyo, Japan) for 5 min. The images were captured using 10 kV of acceleration voltage and 180× magnification.

2.6. In vitro gel digestibility

Digestibility of alginate-corn starch gels was analyzed following the method described by Santamaria et al. (2021) with minor modifications. The amount of alginate-starch gel, freshly prepared, was modified to 200 mg. The enzymes used were porcine pancreatic α -amylase (0.9 U/mL) and amyloglucosidase (143 U/mL). Glucose was determined using a glucose oxidase–peroxidase (GOPOD) kit (Megazyme, Dublin, Ireland). Starch fractions based on the hydrolysis rate were calculated as glucose (mg) \times 0.9 and expressed as glucose content (g/100 g gel) using the method of Kim et al. (2021). Starch fraction hydrolyzed within 20 min of incubation was defined as rapidly digestible starch (RDS), the fraction hydrolyzed within 20 min and 120 min was identified as slowly digestible starch (SDS). Moreover, total digestible starch (DS) and resistant starch (RS) were assessed as hydrolyzed and remnant starch after 24 h of incubation, respectively. The *in vitro* digestion kinetics were fitted by first order equation, Equation (4), based on the method of Ratnaningsih et al. (2017):

$$C = C_{\infty}(1 - e^{-kt}) \quad (4)$$

where, C was the concentration at t time, C_{∞} was the maximum hydrolysis, k was the kinetic constant and t was the digestion time.

2.7. Statistical analysis

Experimental data were statistically analyzed by IBM SPSS statistics V27 (SPSS Inc., Armonk, NY, USA, 2020) software. Data was subjected to analysis of variance (ANOVA) and values were expressed as a mean \pm standard deviation. Duncan test was used to estimate significant differences among experimental mean values with a significance level ($p \leq 0.05$).

3. Results and discussion

3.1. Water retention capacity (WRC)

WRC_{CS} was 0.97 ± 0.04 (g water/g d.b.). To calculate the WRC_{SA} , Equation (2), WRC of starch-alginate mixtures was measured. These WRC values were higher than WRC_{CS} , due to alginates retained relevant amounts of available water in formulations (Hasatsri et al., 2018). The WRC of commercial sodium alginates was 12.09 ± 1.08 and 10.16 ± 2.37 (g water/g d.b.) for P and S. For alginates from the laboratory the WRC values were 7.31 ± 0.61 , 5.26 ± 0.88 and 3.77 ± 0.18 (g water/g d.b.) for ND, 50D and 90D, respectively. For these last alginates, a linear relationship ($R^2 > 0.99$) between WRC and M_v was found, meaning that hydrophilic character increases with the length of alginate polymers. However, this trend was lost when commercial alginates were considered, indicating that source, other polymer characteristics (average block length, mannuronate-guluronate ratio, etc.) and extraction

procedure can strongly affect the physicochemical alginate features.

3.2. Rheological properties

The rheological properties of the starch gel and alginate-starch gels were studied by small amplitude oscillatory shear tests. All experiments were carried out inside LVR previously determined by strain sweep tests. During the formation of the gels, cooling and maturation, the elastic modulus (G') was greater than viscous modulus (G'') giving evidence of a solid-like behaviour. After 10 min at 95 °C all gels achieved constant G' and G'' values, indicating the presence of fully formed gels at this elapsed time. During cooling a sharp increase of G' (7-fold, from 95 to 37 °C) together with a lower increase of G'' (2-fold) were determined. These results were related to gradual gel strengthening (firmer gels) during temperature decrease. Both moduli slightly raised during the first 20 min during maturation at 37 °C, above this period remained invariant. Fig. 1a, as example, shows the G' changes during 30 min of maturation. In fact, the increases of both moduli were proportional and, consequently, $\tan \delta$ varied in a narrow range during maturation, Fig. 1b.

In the Fig. 1 it can be also observed the effect of alginates addition on rheological properties of tested corn starch gels. At the beginning and at the end of maturation period, the presence of alginate at 1% w/w decreased significantly ($p > 0.05$) G' values regarding CS. Nevertheless, when higher amount of alginate was added no significant differences ($p > 0.05$) were observed between G' in samples with 1 or 2% w/w of SA, excepting with P alginate where G' was invariant regarding CS. In general, $\tan \delta$ values were very low (elastic behaviour was very predominant) and slightly varied (0.04 ± 0.01) with the presence of alginates, excepting in the cases of P and S at 1% w/w where higher values (>0.06) were found. These results indicated that former gels were some less structured than the others.

The rheological characteristics of gels formed with alginates from the same seaweeds and with different M_v (ND, 50D, and 90D samples) did not show significant differences between them. These results indicated that M_v of alginate was not critical for the rheological feature of starch gels when alginates had similar nature and extraction procedures. Nevertheless, significant differences were found when alginates from different sources were employed. Fig. 2 shows the frequency sweeps, within the linear viscoelastic region (LVR), performed from 0.1 to 10 Hz at a constant strain of 1% and at 37 °C after maturation period of the tested gels. Gels showed a solid-like behaviour ($G' > G''$) over the whole frequency range, and both moduli exhibited a slight increase with increasing frequency, typical behaviour of weak gels (Ji et al., 2017). In Fig. 2a, G' values are shown for the CS sample and samples with 1% w/w of alginate. It was observed that, with the addition of alginate, G' decreased over the whole frequencies range, and consequently the gels rigidity. It is worthy to note that two groups of data were clearly visible: those with the addition of commercial alginates (P and S), and those gels formed after the addition of in-house isolated alginates with different M_v . In the former no significant differences between respective trends with angular frequencies were found, but showed lower G' than the gels with commercial alginates within the frequency range. When more amount (2% w/w) of commercial alginate was added, Fig. 2b, G' values were systematically higher than those obtained after alginate addition at 1% w/w. Even with P alginate addition, G' values were similar to those observed with CS gel at low frequencies (<1 Hz) and above G' values were slightly higher. Nevertheless, in-house isolated alginates addition decreased G' dramatically and the drop was practically invariant with the added amount of alginate. This fact could be related to the low WRC values of these alginates in comparison to commercial ones. The addition of alginate at 1% w/w clearly increased $\tan \delta$ values due to the enhancement of viscous character of these gels, Fig. 2c. In fact, the greatest increase of $\tan \delta$ was due to the addition of the alginate with the highest M_v . At high (2% w/w) addition of alginates, Fig. 2d, all samples, at low frequencies, showed similar trend, and viscous character increased at high angular frequencies when SA was added. Therefore,

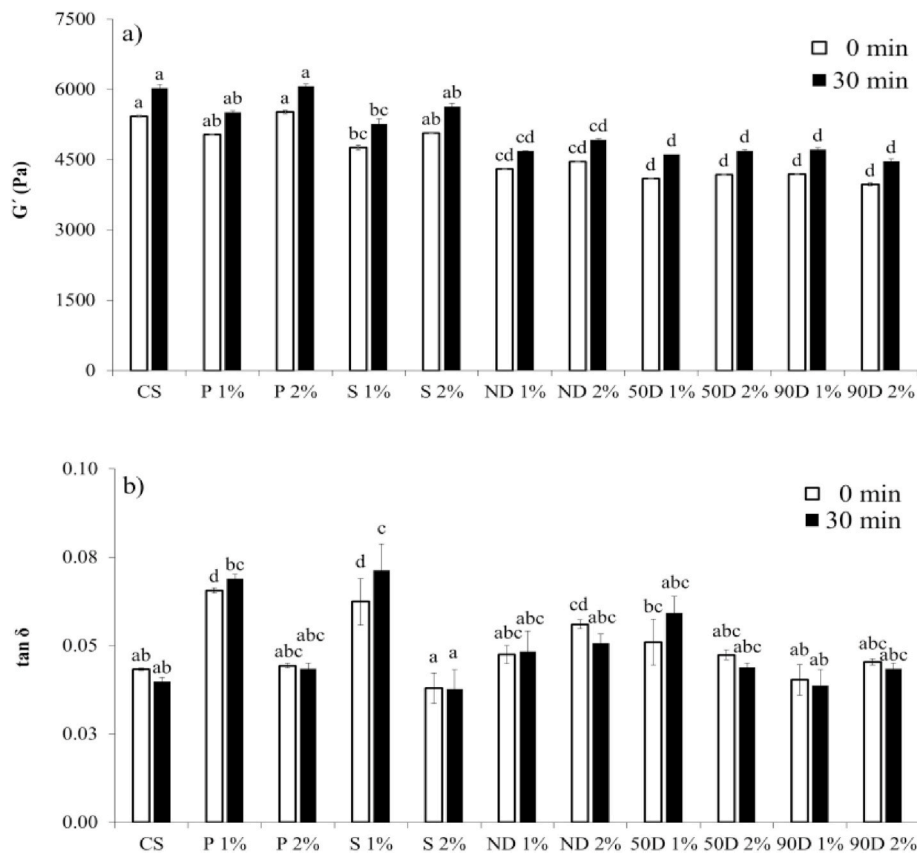


Fig. 1. G' (a) and $\tan \delta$ (b) values at the beginning (0 min) and at the end (90 min) of maturation period at 37 °C measured at 30 Pa and 1 Hz of corn starch (1:4) gel (CS), CS with commercial alginates: PanReac (P) and Sigma (S), and with processed alginates: not dried (ND), dried at 50 °C (50D), dried at 90 °C (90D) at 1 and 2% (w/w). Different letters on bars denote significant differences ($p < 0.05$).

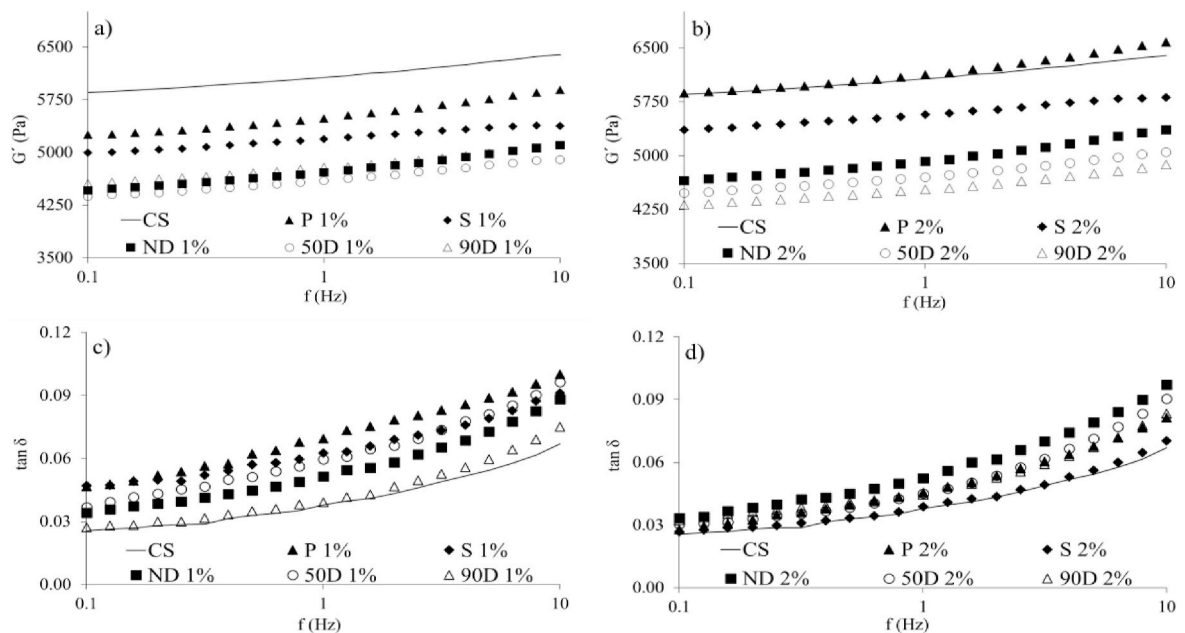


Fig. 2. Frequency sweep tests G' (a, b) and $\tan \delta$ (c, d) of corn starch (1:4) gel (CS), CS with commercial alginates: PanReac (P) and Sigma (S), and with processed alginates: not dried (ND), dried at 50 °C (50D), dried at 90 °C (90D) at 1% (a, c) and 2% (b, d) alginate content.

alginate addition did not promote the gelation and short time retrogradation of starch by means of additional crosslinking bonds among molecules, and consequently weaker structures were formed, and in turn viscous character was more relevant. In fact, Li et al. (2017) reported

that early stage of retrogradation given by the aggregation of amylose was significantly retarded because alginates were associated to leached amylose. Other authors found similar trends (G' decreased and $\tan \delta$ increased) with SA addition; in example, Fang et al. (2020) added

sodium alginate (1% w/w starch basis) to waxy potato starch gels (10% w/w) and He et al. (2019) studied rice starch (10% w/w) with sodium alginate (5% w/w starch basis). However, Yang et al. (2021) found that both moduli, G' and G'' , increased with SA content employing rice starch at lower content (5% w/w) and alginates in the range 0.1–0.5% (w/v).

Complex modulus, G^* , of all tested systems was successfully fitted ($R^2 > 0.97$) to Equation (3) and values of parameters are given in Table 1. Coordination number, z , corresponding to CS and S (independently of SA amount added) showed significantly higher values (from 51.52 to 52.76) with respect to samples with other SA (from 31.47 to 38.99). These results indicated that relevant structural changes occurred by the addition of some alginates (P, ND, 50D and 90D) regarding CS gel and the number of rheological units (less compact 3-D network of gel) decreased significantly with the presence of these alginates. Moreover, z was invariant ($p < 0.05$) with the SA content in all cases. No significant differences among alginates with same origin and obtained under the same processing (ND, 50D, and 90D) were observed. In relation to A parameter, a significant decrease ($p > 0.05$) with alginates addition were observed regarding CS. Nevertheless, in this case, some significant increase of A with SA amount could be determined. In fact, like all samples showed a predominant elastic behaviour ($G' \gg G''$), the previous discussion about G' trends is also valid here. These results again seemed to indicate that both different source and production process of alginates were more critical than the M_v value to change the starch-gel structure.

On the other hand, the increase of elasticity with increasing alginate concentration could be expected due to lower available water for gel formation by the increase of total dry matter and high WRC of alginates. Interestingly, G' values (measured at 1 Hz and 1% strain) of alginate-starch systems were related to WRC_{SA} values. At high alginate addition (2% w/w), an acceptable positive linear regression was established ($R^2 > 0.98$) between WRC_{SA} and the relative drop of G' for alginate-starch gels regarding pure starch gel, Fig. 3. Curiously, when alginates from AN with different M_v , whose nature and extraction method were controlled, are only considered, $R^2 = 1$ was obtained. Performing the same analysis at low alginate content (1% w/w) a worse linear regression ($R^2 < 0.8$) was achieved. These results could indicate that alginates at low content interacted with leached amylose in different extension as function of their specific molecular characteristics that also determined their water retention. However, at high alginate content the interactions with amylose were less relevant regarding water held by alginates and gel rigidity depended straight on WRC_{SA} .

3.3. Microstructure of alginate-corn starch gels

The impact of alginates addition on the starch gel's structure was analyzed. Fig. 4 shows some micrographs, as example, of tested gels. CS, Fig. 4a, presented uniform and circular structure; but the addition of

Table 1
Values of parameters of Equation (4).

Samples	A (kPa)	z
CS	6.07 ± 0.66 ^{ab}	51.52 ± 1.06 ^a
P 1%	5.52 ± 0.01 ^c	36.96 ± 0.63 ^{bc}
P 2%	6.16 ± 0.53 ^{ab}	38.99 ± 2.52 ^{bc}
S 1%	5.20 ± 0.26 ^{cd}	52.47 ± 3.56 ^a
S 2%	5.58 ± 0.21 ^{bc}	52.76 ± 3.93 ^a
ND 1%	4.74 ± 0.51 ^{de}	33.81 ± 2.36 ^c
ND 2%	4.95 ± 0.39 ^{de}	31.47 ± 0.79 ^c
50D 1%	4.62 ± 0.37 ^e	37.43 ± 1.16 ^{bc}
50D 2%	4.73 ± 0.15 ^{de}	37.22 ± 1.61 ^{bc}
90D 1%	4.63 ± 0.21 ^e	38.88 ± 3.93 ^{bc}
90D 2%	4.55 ± 0.10 ^e	37.89 ± 2.88 ^{bc}

Corn starch (1:4) gel: CS, CS with commercial alginates: PanReac, P, and Sigma, S; and with processed alginates: not dried, ND, dried at 50 °C, 50D, dried at 90 °C, 90D at 1 and 2% (w/w). Values followed by different letters within a column denote significant differences ($p < 0.05$).

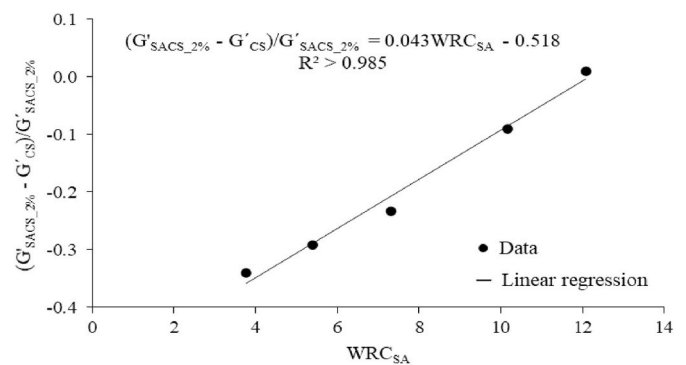


Fig. 3. Linear regression between (G' (alginate 2% w/w) – G' starch)/ G' (alginate 2% w/w) and water retention capacity of alginate (WRC_{SA}).

alginates, Fig. 4b–e, modified clearly the microstructure creating elongated and unequal cavities, described as “honeycomb” network structure. In all cases, the structure of alginate-starch gels was formed by long linear partition walls that configured spatial anisotropic networks. It has been described that the effect of hydrocolloids on starch properties depend on the hydrocolloid structure and concentration, giving weaker structures and less gel-like behaviour due to interactions between starch and hydroxyl groups (Liu et al., 2022). Likely, the interaction with the incorporation of SA was produced at the beginning of starch gelatinization, which could alter the microstructure. Moreover, hydrocolloid concentration could be associated to a phase separation process. Rosell et al. (2011) related this effect to the formation of a network structure with fewer bonds among the starch and more entanglements between the hydrocolloid's chains. This could describe the elongated structures observed after the addition of SA. Fig. 4b and c, for P alginate, as example, show that structure was maintained invariant with increasing SA content. Probably a threshold alginate content (less than 1% w/w) was necessary to create the described structures. Fig. 4d and e shows the micrographs of alginate-starch gels with ND and 90D (high and low M_v) and no significant differences were found between them (independently of SA addition). Nevertheless, in both gels (also with 50D), in comparison to P gels, bigger cavities and more space between partition walls could be observed and this fact could explain their weaker elastic character. Liu et al. (2021) also observed structural changes, employing chestnut starch gels, with fewer and more irregular pores in alginate-starch gels with higher SA amount added. This modification in the microstructure could be also related to water mobility, being this minimized when increasing the amount of SA.

3.4. In vitro digestion of starch gels

Typical hydrolysis profiles, Fig. 5a and b, were obtained for commercial and processed alginates, with a sharp increase of hydrolyzed starch during the first 20 min (in the slowest kinetics) up to achieve a stationary content after 60 min (also in the slowest digestion). Hydrolysis plots depended on the type of alginate and their concentration. Globally, the presence of alginates slowed down the hydrolysis rate of starch gels and, in general, increasing alginate content the kinetics rate decreased, Fig. 5a, as example. Fig. 5b shows the same slow hydrolysis kinetics of starch gels with low M_v (S and 90D) alginates at 2% w/w and the clear increase of the hydrolysis extent of corn starch. Other authors also reported that the alginate content was a critical factor affecting starch hydrolysis. Jung et al. (2017) suggested 0.5% as the optimal concentration of hydrocolloids, for affecting the predicted glycemic index. Moreover, Ramírez et al. (2015) reported a significant decrease of potato starch hydrolysis when adding alginate (1.0 and 2.0 g/100 g), which was attributed to change in the viscosity of starch solutions. Hydrolysis parameters extracted from the plots confirmed the impact of alginate addition (Table 2). Starch fractions SDS, DS and RS were

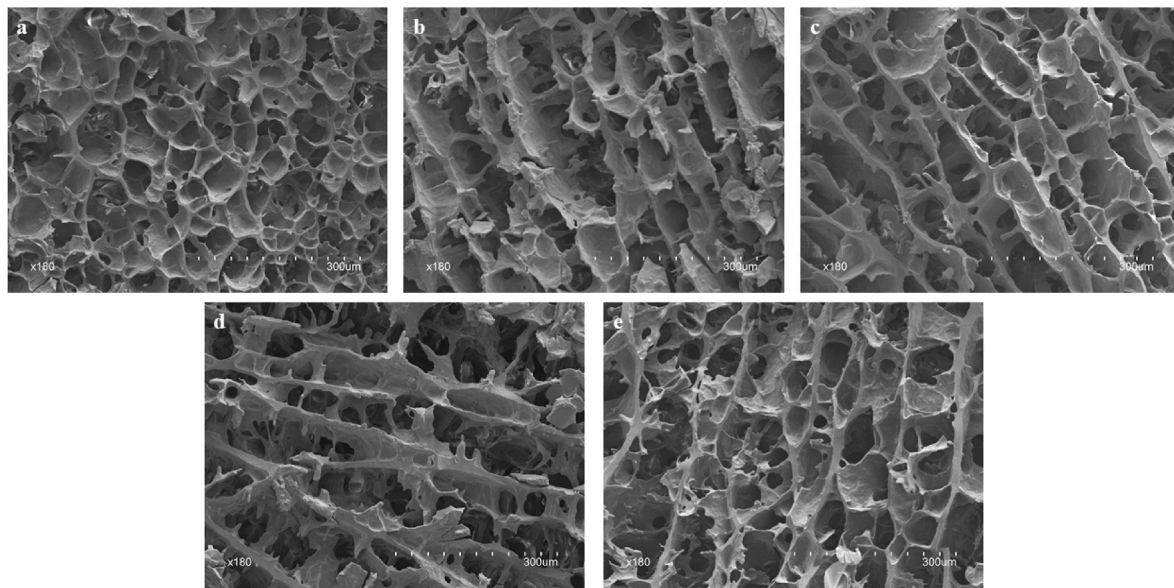


Fig. 4. Micrographs of alginate-corn starch gels. Magnification 180x. (a) Corn starch (CS); (b) and (c) Commercial alginate: PanReac (P) at 1 and 2% w/w starch basis, respectively; (d) processed alginates: not dried (ND) and (e), dried at 50 °C (50D) at 1% w/w, and 90 °C (90D) at 2 w/w.

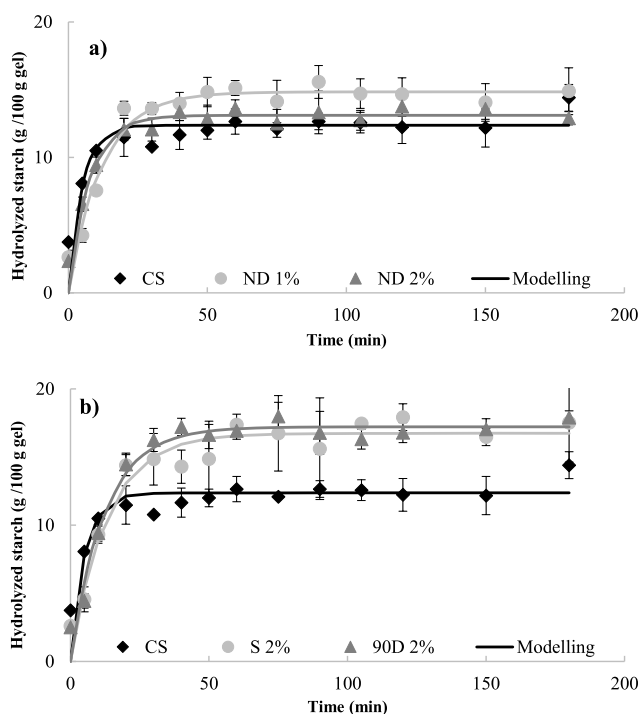


Fig. 5. Enzymatic hydrolysis plots of alginate-corn starch gels. (a) Effect of not dried alginates (ND) at 1 and 2% w/w. (b) Effect of commercial and processed alginates (2% w/w): Sigma (S) and dried at 90 °C (90D). Hydrolysis of corn starch (CS) was included in both graphs. Modelled data by Equation (4).

significantly modified depending on the type of alginate. RDS varied in a narrow interval from 10.65 (S 1%) to 13.80 g/100 g (90 D 2%). RDS was maintained constant or increased significantly with SA content, but no clear trends according to SA type or M_v were observed (Table 2). Conversely, at 1% of SA content, SDS increased significantly ($p > 0.05$) with respect to control (CS) from 0.25 up to 2.85 g/100g (except for 50D addition where no significant differences were observed). SDS significantly increased (up to 3.67 g/100g with S 2%) with added SA amount but decreased with increasing ND and 50D additions. These results could

be related to commercial SA might be subjected to thermal processes or solvent extraction that modified their features, facilitating the interaction with the starch chains, behaving like 90D. In opposition, ND and 50D directly extracted by mild physical methods interacted with starch at low concentrations, but higher ones facilitate their inner interactions that led to the phase separation. DS increased in all SA containing gels, except for S 1% and 50D that showed values close to CS. Finally, RS also varied in an interval from 1.36 (50D 2%) up to 2.58 (90D 2%) (g/100 g) with significant differences between alginates employed. These findings were also partly observed by Chung et al. (2007) in cooked rice where SDS content increased, and RS reduced with the SA addition.

Equation (4) was successfully, Fig. 5a and b, employed to hydrolysis kinetics modelling. Hydrolysis kinetic constant (k) decreased with the alginate addition to corn starch gels, except 50D at 2% w/w. Hydrocolloids have been described to act as a barrier between the enzymes and starch molecules, in consequence retarded starch digestion. Feng et al. (2019) observed that the incorporation of xanthan and sodium alginate reduced the starch digestibility of pasta and noodles. When the hydrocolloids were mixed with starch, these interact with leached amylose and amylopectin with low molecular weight developing different network structures. Jung et al. (2017) related the reduced hydrolysis rate with the inhibition of starch swelling, due to the incorporation of arabic gum. In general, this effect was improved at high SA content (2% w/w) and kinetic constant decreased, particularly, with commercial alginates because they increased the rigidity of gels by their high hygroscopic character. With the remaining less hygroscopic alginates this effect was not observed. Maximum hydrolysis (C_∞) increased with SA addition from 12.37 (CS) up to 17.22 (90D 2%) due to mainly the described increase of SDS fraction. No clear trends of C_∞ with the presence of alginates and with added amount of SA were found, but the increase of digestibility could be related to the specific interactions between employed SA with amylose and amylopectin that decreased the starch retrogradation facilitating the access of enzyme during long digestion time and could be responsible of the DS, SDS and C_∞ results obtained with the SA addition. The different interactions of SA with starch might be also responsible of the RS results. However, it was not possible to correlate RS content with the molecular weight or SA content. Additional research is required to understand the entanglements of starch in the presence of different SA. In brief, slower hydrolysis kinetics could be related to the interference of SA molecules in the starch-enzyme interaction and the higher amount of hydrolyzed starch could be

Table 2
Parameters of *in vitro* alginate-corn starch gels digestion: rapidly digestible starch (RDS), slowly digestible starch (SDS), digestible starch (DS), resistant starch (RS), kinetic constant (*k*) and maximum hydrolysis (C_{∞}).

Sample	RDS (g/100 g)	SDS (g/100 g)	DS (g/100 g)	RS (g/100 g)	<i>k</i> (min ⁻¹)	C_{∞}
CS	12.13 ±	0.25 ±	11.85 ±	2.06 ±	0.20 ±	12.37 ±
P 1%	13.47 ±	0.56 ±	14.73 ±	2.40 ±	0.16 ±	14.03 ±
P 2%	13.70 ±	1.28 ±	13.98 ±	1.77 ±	0.12 ±	14.98 ±
S 1%	10.65 ±	1.79 ±	12.60 ±	1.70 ±	0.10 ±	12.45 ±
S 2%	13.07 ±	3.67 ±	13.95 ±	1.70 ±	0.08 ±	16.74 ±
ND 1%	11.99 ±	0.35 ±	14.76 ±	2.11 ±	0.08 ±	14.84 ±
ND 2%	12.13 ±	0.98 ±	14.55 ±	2.25 ±	0.13 ±	13.11 ±
50D 1%	13.43 ±	0.21 ±	12.67 ±	1.48 ±	0.17 ±	13.64 ±
50D 2%	13.27 ±	0.05 ±	12.69 ±	1.36 ±	0.26 ±	13.32 ±
90D 1%	11.33 ±	1.41 ±	13.78 ±	2.16 ±	0.11 ±	12.74 ±
90D 2%	13.80 ±	3.41 ±	14.64 ±	2.58 ±	0.08 ±	17.22 ±
<i>P-Value*</i>	0.14	0.01	0.01	0.01	0.01	0.73
Alginate Concentration	0.04	0.21	0.39	0.75	0.98	0.06

Corn starch (1:4) gel: CS, CS with commercial alginates; PanReac, P, and Sigma, S; and with processed alginates: not dried, ND, dried at 50 °C, 50D, dried at 90 °C, 90D at 1 and 2% (w/w). Values followed by different letters within a column denote significant differences ($p < 0.05$). *k* and C_{∞} were determined by the Equation (3), $C = C_{\infty}(1 - e^{-kt})$. *The significance of the two factors (alginate type and their concentration) after the statistical analysis is indicated.

explained by the structural changes and previously described together with the presence of less retrograded starch amount that promote the accessibility of the enzyme to the starch. This also could be associated with a phase separation process, as mentioned above, with increasing SA content that favours more entanglements between the hydrocolloid's chains (Rosell et al., 2011). SA addition affected significantly enzymatic hydrolysis due to changes among molecular interactions.

4. Conclusions

The addition of sodium alginates caused relevant changes in the viscoelastic characteristics and structural features of the corn starch gels with presence of elongated and unequal cavities and the formation of linear partition walls that configured spatial anisotropic network. These structures were maintained independently of the tested alginate content. The addition of alginates with different origin and production method significantly modified specifically the rheological properties. Weaker gels (lower elastic modulus) were obtained with the addition of alginates and viscous character increased. Stronger gels were obtained increasing alginate content when hydrophilic character of alginates was high. The hydrolysis constant decreased with the addition of any alginate and, in general, with the amount of alginate with high hygroscopicity. At low alginate content, complexes were formed by alginate and leached amylose (and low Mv amylopectin) being hardly hydrolyzed by the enzymes and hindered the enzyme access to the remaining starch for its hydrolysis despite the generation of more open structures. These structures and a less starch retrogradation facilitated the later accessibility of enzymes to starch resulting that both SDS starch fraction and total hydrolyzed starch amount increased. In summary, alginate-corn starch gels can be produced with the same viscoelastic characteristics of corn starch gels, but with different gel structure and lower hydrolysis rates (interesting to decrease glycemic peak). However, in alginate-corn gels with similar structure promoted by the presence of alginates, the increase of the viscoelastic characteristics decreased the hydrolysis rate of starch. That is, the hydrolysis rate depended on rheology and structure of gel.

Contributions of authors

Leticia Montes: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Writing-original draft; Maria Santamaria: Investigation; Methodology; Writing-original draft; Raquel Garzon: Methodology; Supervision; Data curation; Cristina M Rosell: Conceptualization; Funding acquisition; Supervision; Writing - review & editing; Ramón Moreira: Conceptualization; Formal analysis; Writing-review & editing; Funding acquisition.

Declaration of competing interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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