RESEARCH ARTICLE

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Performance of Starch Gels on In Vitro Enzymatic Hydrolysis Assessed by Rheological Methodologies

*Maria Santamaria, Leticia Montes, Raquel Garzon, Ramón Moreira, and Cristina M. Rosell**

Starch hydrolysis is attracting much attention due to its relationship to digestion and glucose release. The objective is to propose rapid and continuous analytical methods that allow measuring gels hydrolysis following apparent viscosity (μ). Three different starches (corn, wheat, and rice) are tested recording starch gelatinization followed by gels digestions (digestograms) using a rapid-visco analyzer (RVA) or a rheometer. Results are compared with those obtained by measuring glucose release along hydrolysis. A modified first-order kinetic model in the RVA ($R^2 > 0.99$) and rheometer ($R^2 > 0.99$) describes the gels digestograms. Wheat gel shows a higher hydrolysis rate (k), which indicates faster digestion followed by rice and corn gels. The proposed models allow rapid analysis of starch digestograms, allowing to discriminate among hydrolysis rate of different starches. These less time-consuming methods can be an option to continuously analyze starch gelatinization followed by enzymatic digestion.

1. Introduction

Nowadays, one of the trend drivers for food manufacturers is the development of healthy foods, particularly addressing increase of nutrient availability, improve satiety, or decrease blood glucose response.[1] Because of that, much interest has been focused on developing in vitro methods that allow predicting foods and nutrients behavior along the oro-gastrointestinal digestion.[2,3] Particularly in the case of starch digestion, the oro-gastrointestinal digestion is rather challenging due to the many dilutions that masked the kinetic changes in the starch fraction.[4] Alternatively, in vitro starch digestion methods are the most applied ones, mainly based on enzymatic hydrolysis followed by measuring the glucose release.[5] However, other indirect methods for assessing starch performance along enzymatic digestion have also attracted attention, particularly following viscosity[6] and the impact of different enzyme concentrations[7] during digestion simulation, initially using a rotary viscometer. Nowadays, there are other equipment commonly used for following rheological changes, namely rheometer and rapid visco analyzer (RVA), and some authors have already used them to record rheology changes that occurred along digestion at 37 °C.[8,9] Other authors followed the glucose release that occurs during the digestion period in parallel to rheology changes recorded in the rheometer.[10–13] In those studies, focus has been put on the impact of shear rate (0.1, 1, 10 s^{-1}) on the in vitro digestion of gelatinized potato and corn starch[12] or the impact of hydrocolloids like guar gum on the digestibility of potato flour[11] or its effect on waxy maize.[13] Hardacre et al.[10] also studied de impact of soluble and insoluble fiber in potato and corn starches during their in vitro digestion.

Similarly, RVA has been used to evaluate the apparent viscosity decay produced on different wheat starch gels (6%, 8%, and 10%) or waxy maize starch gels (2%, 4%, and 6%) at 37 °C when adding different levels of α-amylase and their relationship with volatile compounds release, but without relating those with starch digestion.[8] Conversely, Sorba et al.[9] studied the enzymatic hydrolysis of potato and waxy maize starch gels using amylase and amyloglucosidase and recording apparent viscosity changes with RVA.

Furthermore, Hódsági et al.[14] found some significant correlations among glucose release during enzymatic hydrolysis of corn and wheat starches and their pasting parameters; particularly in the case of wheat starch hydrolysis rate and peak viscosity, trough, and final viscosity, which might be useful for estimating in vitro digestion. However, previous studies have been conducted using rheology methods to independently evaluate gelatinization behavior of starches or to follow rheological modifications during the enzymatic hydrolysis. The aim of this study was to develop rapid methods that allow in a single test to evaluate starch performance during gelatinization followed by enzymatic digestion. For that purpose, rheological methods were developed in the RVA and rheometer using α-amylase, and result compared with the data obtained by quantifying glucose release. The inclusion

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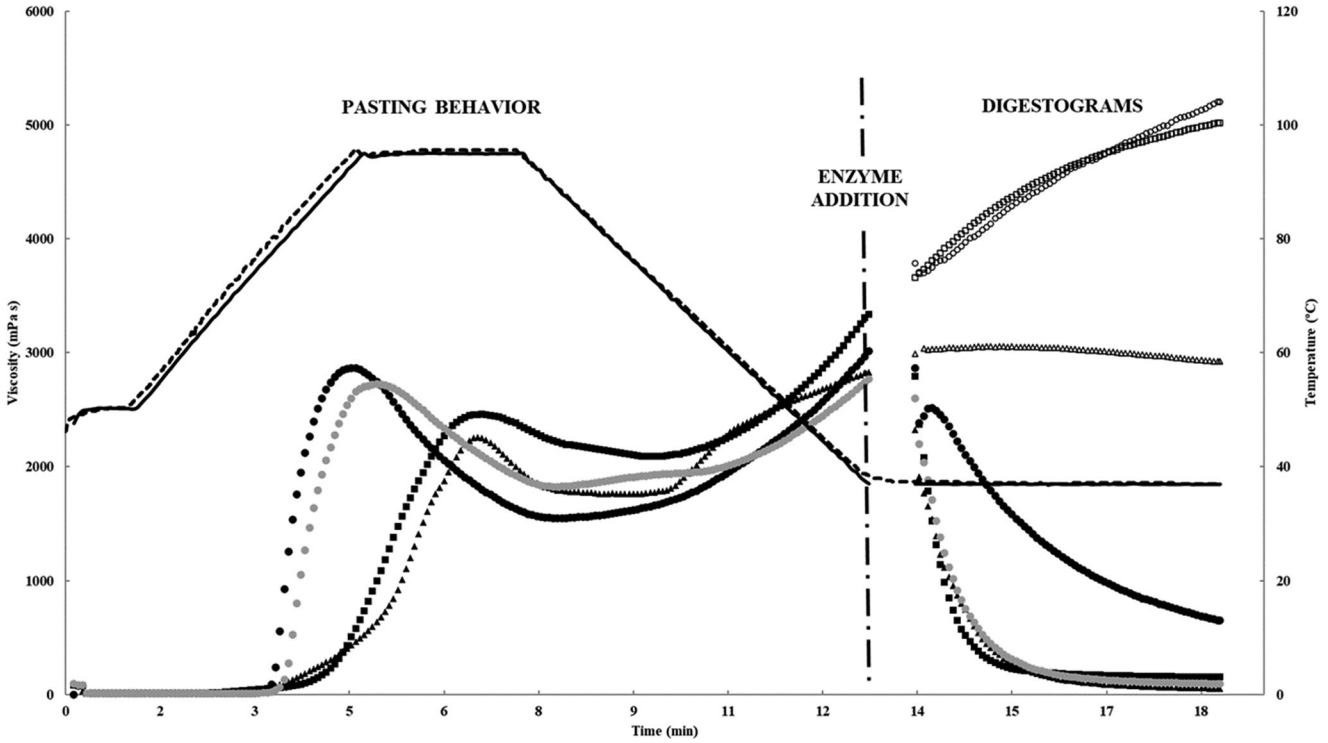

Figure 1. RVA method for recording the starch gelatinization and further enzymatic hydrolysis. First part records the pasting behavior of the gels, then the addition of alpha-amylase and finally the digestograms in the presence of amylase (filled symbols) and their counterparts in the absence of enzyme (empty symbols). Corn (●), corn pH 5.8 (●), wheat (■), and rice (▲) starches. Theorical (▬) and experimental (-----) temperatures (°C).

of enzymatic hydrolysis into the rheological methods might provide rapid methods to predict the behavior of starch gels during enzymatic digestion.

2. Results and Discussion

2.1. Viscosity Hydrolysis

Corn, wheat, and rice starches were selected to set up a rapid method for assessing pasting performance followed by enzymatic hydrolysis in a single assay, which were referred as digestograms. Plots of the apparent viscosities along pasting and enzymatic hydrolysis are shown in **Figure 1**. Parameters recorded from the apparent viscosity plots are indicated in Table S1, Supporting Information. Knowing the importance of temperature on the enzymatic kinetics, thermocouples were immersed in the slurries to monitor it, and values completely overlapped those recorded by the equipment. As expected, the apparent viscosity plots for corn, wheat, and rice indicate differences in their pasting performance, with corn showing an earlier swelling and major maximum apparent viscosity (2866 ± 15 mPa s) than observed in the other starches, which agree with previously reported results.[4] Moreover, Wickramasinghe et al.[15] observed different viscosity peaks and swelling power among several varieties of hard or soft wheat starches. Rice showed lower apparent peak viscosity (2263 ± 93 mPa s), with similar value to the one reported.[16] Starch granules differ in morphological, and starch structure depending on botanical origin, which affect their pasting performance.[17]

Focusing on the hydrolysis or digestogram stage, apparent viscosities of the gels in the presence and the absence of α-amylase were recorded. In the absence of α-amylase (empty symbols) a progressive increase in the apparent viscosity was observed in corn and wheat gels. Presumably, that increase in the apparent viscosity was related to their slower cooling due to their higher viscosity, which reduced the cooling rate within the gel structure. In fact, in the case of rice gel, a steady apparent viscosity was observed because its lower viscosity allowed faster heat transference within gel structure. The addition of α-amylase produced a rapid decline in the apparent viscosity, similar to that observed Gee et al.[6] using a rotary viscometer. Enzymatic hydrolysis by α-amylase induces the breakdown of starch chains to the release of small fragments (dextrins) changing the starch gel behavior, from a solid gel to a weakly structured fluid gel.[9] Nonetheless, comparing the digestograms of the different starches, corn gel showed lower viscosity decrease (2864–651 mPa s) (**Table 1**). Considering the impact of pH on the enzymatic activity, first hypotheses was related to possible pH difference.[18] In fact, corn starch slurry had pH 7.25, whereas slurries of wheat and rice starches showed pH 5.85. To confirm the impact of gel pH on α-amylase activity, corn starch gel was prepared in sodium phosphate buffer 0.01 M at pH 5.8 instead of water. The digestogram obtained for corn gel with adjusted pH displayed faster hydrolysis, like the one obtained with wheat and rice gels.

Gels formation and their further hydrolysis were also carried out in the rheometer. In **Figure 2** it can be observed the formation of the gels and then, its maturation (empty symbols) and

Table 1. Gel starch viscosities (μ) obtained with RVA or rheometer before and after adding amylase, and the parameters that defined the hydrolysis kinetic (the kinetic constant and the maximum hydrolysis of starch gels).

Method	Parameters	Corn	Corn pH 5.8	Wheat	Rice
RVA	μ initial digestion [mPa s]	2864 ± 90^{a}	2599 ± 146^{ab}	2793 ± 183^{a}	2324 ± 106^{b}
	μ final digestion [mPa s]	651 ± 4^{a}	96 ± 10^{c}	154 ± 8^{b}	54 ± 3^{d}
	k_{RVA} [min^{-1}]	0.40 ± 0.06^{c}	1.33 ± 0.12^{b}	1.80 ± 0.02^{a}	1.17 ± 0.11^{b}
	μ_{∞} [mPa s]	329 ± 41^{a}	75 ± 4^{c}	137 ± 8^{b}	34 ± 6^{c}
Rheometer	μ initial digestion [mPa s]	4975 ± 78^{a}	4670 ± 269^{ab}	4520 ± 14^{b}	2445 ± 134^{c}
	μ final digestion [mPa s]	1810 ± 42^{a}	686 ± 15^{b}	323 ± 26^{b}	94 ± 17^{c}
	k_{Rheo} [min^{-1}]	0.46 ± 0.01^{d}	0.74 ± 0.08^{c}	2.38 ± 0.07^{a}	1.04 ± 0.02^{b}
	μ_{∞} [mPa s]	1549 ± 68^{a}	677 ± 114^{b}	336 ± 50^{c}	83 ± 16^{d}
Biochemical	k [min^{-1}]	0.0334 ± 0.0009	–	0.0399 ± 0.0049	0.0335 ± 0.0012
	C_{∞} [g 100 g^{-1} gel]	6.38 ± 0.35^{ab}	–	5.51 ± 0.24^{b}	6.97 ± 0.55^{a}

Means within a row followed with different letters indicate significantly different ($p < 0.05$).

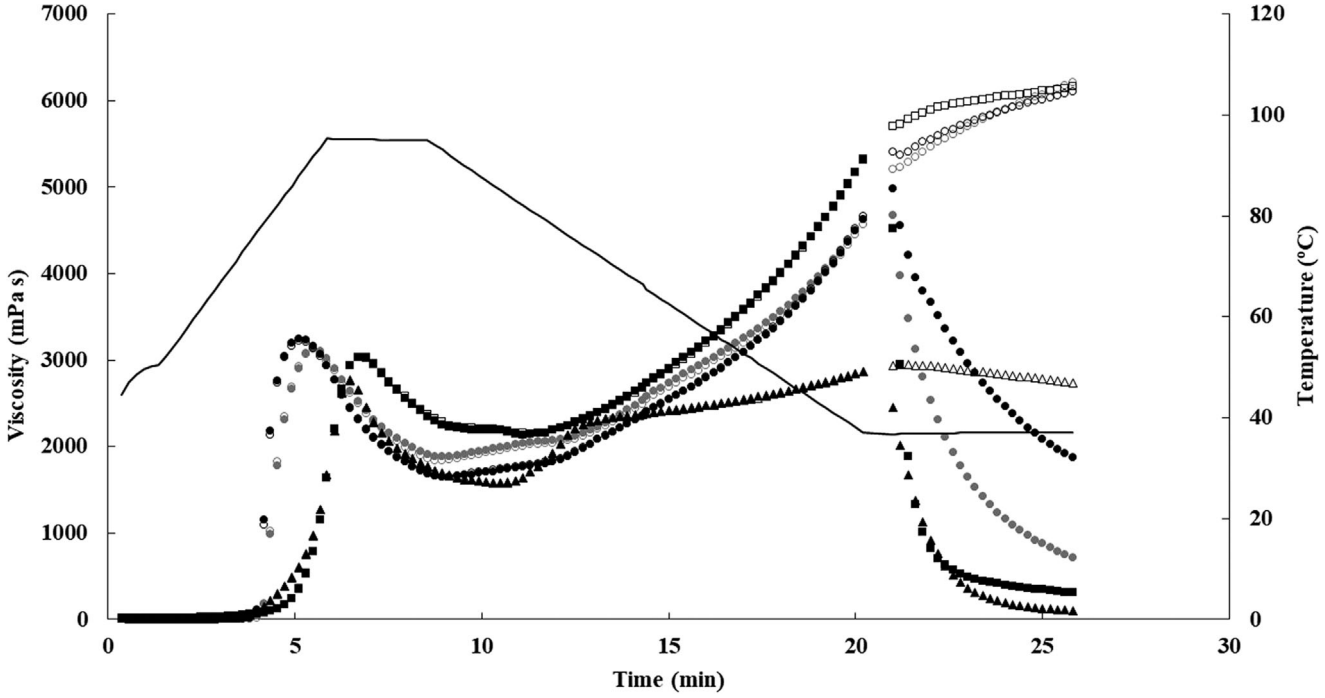

Figure 2. Full assay in rheometer where it is represented the apparent viscosity versus time following the protocol previously described for corn (●), corn pH 5.8 (●), wheat (■), and rice (▲) starches.

digestion (filled symbols). In general, same behavior than in RVA assays was observed. At the end of the gelatinization stage, it was observed that wheat starch had the highest viscosity (4520 ± 14 mPa s), while rice starch presented the lowest viscosity (2445 ± 134 mPa s) (Table 1). At digestion stage, a significant decrease in viscosity was seen in all samples, which agrees with results obtained with the RVA. Similar behavior was previously reported by Kim et al.[19] when simulated the oro-gastrointestinal digestion of white and brown rice flours in the rheometer, and An et al.[20] also reported a decrease of viscosity when wheat gels blended with increasing amounts of black rice flour were digested with pancreatin and amyloglucosidase.

2.2. Enzymatic Hydrolysis of Different Starches Recorded by Biochemical Methods

Starch gels obtained from RVA were subjected to in vitro digestibility to evaluate the hydrolysis kinetics of starches from different cereals, and to compare those with the results obtained in the rapid methods previously presented. In **Figure 3** hydrolysis plots of gels are displayed. The graphs were expressed as grams of hydrolyzed starch per 100 g of gel. Hydrolysis pattern was different among the starches from different botanical origin. Rice gel presented higher hydrolysis, which could be related to its lower initial viscosity (2263 mPa s) that facilitates

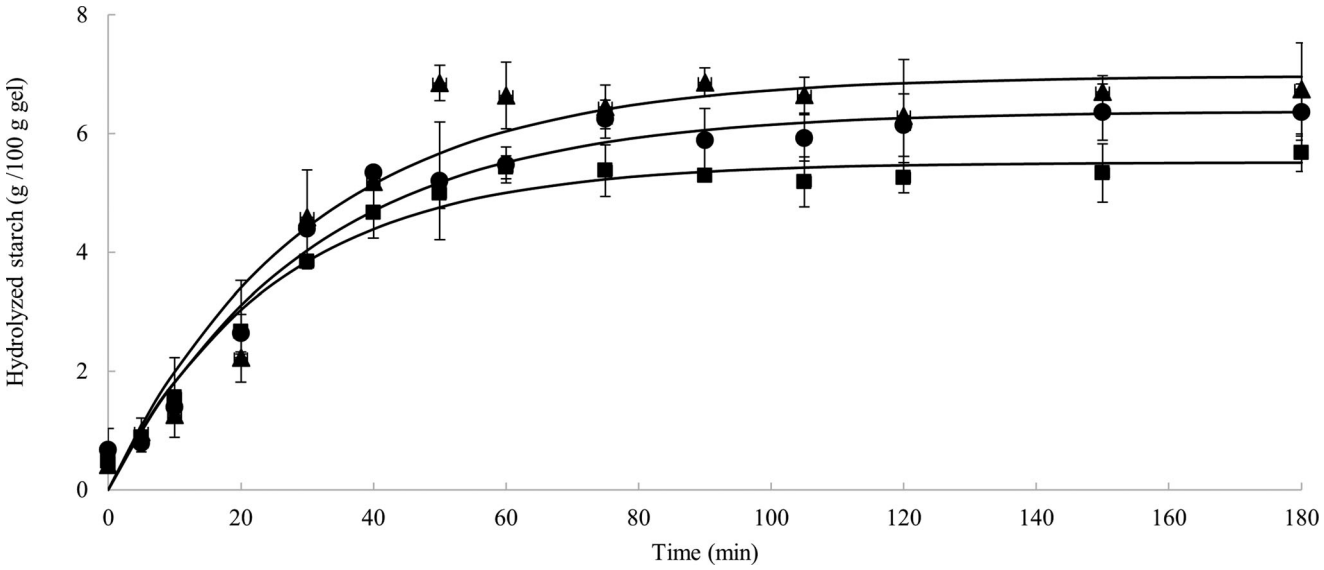

Figure 3. Enzymatic hydrolysis of different starch gels corn (●), wheat (■), and rice (▲) starches, and solid lines correspond to first-order model Equation (3) (▬).

enzyme diffusion (Table S1, Supporting Information).[21] Consequently, rice gel reached the superior maximum hydrolysis (C_∞) (Table 1). Kinetics parameters were satisfactorily fitted ($R^2 > 0.96$) with a fist-order kinetics-based model Equation (3). Gels presented similar hydrolysis rate (k) and differed in the extent of the hydrolysis (C_∞), with rice gel having the highest maximum hydrolysis (Table 1). Hódsági et al.[14] reported similar rate constants for gelatinized wheat and corn starches. Furthermore, although there were not significant differences, gels with lower k had higher slowly digestible starch (SDS) content. This fraction of starch is associated with satiety, less glycemic index, and prebiotic effect.[22]

2.3. Modeling of Digestograms

To establish the correlation between enzymatic hydrolysis of starches by assessing glucose release and the viscosity decay measured either with RVA or rheometer, experimental data of the digestograms were mathematically fitted. **Figure 4** shows the starch hydrolysis by viscosity decay of gels of corn, wheat, and rice starches. The shapes of the kinetics curves were similar, but the initial (related to initial gel firmness) and final viscosities were specific for each starch. In fact, experimental apparent viscosity (mPa s) at the beginning and end of the digestograms obtained in the RVA differed from 2864 to 651 for corn without pH adjustment, 2599–96 for corn at pH 5.8, 2793–154 for wheat, and 2324–54 for rice (Figure 4A). Likewise, digestograms in the rheometer show that apparent viscosity (mPa s) varied from 4975 to 1810 for corn, 4670–686 for corn pH 5.8, 4520–323 for wheat, and 2445–94 for rice starch gels (Figure 4B).

A first-order kinetic model was applied to model the digestograms, Equation (1):

$$\mu = \mu_\infty + \left(\mu_0 - \mu_\infty\right) e^{-kt} \quad (1)$$

where μ is the apparent viscosity (mPa s), μ_0 is the initial viscosity, μ_∞ is the final viscosity, k (min^{-1}) is the kinetic constant, and t (min) is hydrolysis time.

The RVA experimental data presented satisfactorily fitting ($R^2 > 0.99$) to first-order kinetic model. Kinetic constant (k_{RVA}) obtained in the digestograms presented statistically differences ($p < 0.05$) depending on the starch source, as well as pH, in the case of corn starch (Table 1). The highest hydrolysis rate (k_{RVA}) was presented by wheat gel (1.80 min^{-1}), followed by corn gel after adjusting pH (1.33 min^{-1}), and rice (1.17 min^{-1}). Corn gel prepared without adjusting the pH showed the lowest k_{RVA}. Regarding μ_∞, the lowest value was determined for rice starch (34 mPa s) and the highest with corn (329 mPa s). Higher peak viscosity has been correlated negatively with hydrolysis rate of native starches, but no correlations were observed with the enzymatic hydrolysis of the gels.[23] Factors like source starch, enzyme type, concentration of enzyme, and starch solids content affect the starch digestion rate.[9]

Similar fitting was carried out with the experimental data obtained with the rheometer (Table 1) obtaining significant differences ($p > 0.95$) between k_{Rheo} and μ_∞ values for each gel were found. In Figure 4B, it can be observed the acceptable fitting quality ($R^2 > 0.99$) of the model in comparison to experimental data. Again, corn gel without adjusting the pH showed the lowest k_{Rheo} value (0.46 min^{-1}) and wheat the highest (2.38 min^{-1}). Considering the kinetics rate obtained in the RVA, the k_{Rheo} for corn gel at pH 5.8 was lower than expected, even lower than that obtained for rice. Likely differences between rotational speed of rheometer and shearing of RVA, might explain that trend. Presumably, pH equilibration of gel slurry and the enzymatic solution by the employed impellers occurred at different speed in both equipments. The slower homogenization in the rheometer would explain the lower kinetic constants obtained for corn at pH 5.8 versus rice value, in comparison with their respective RVA results. Nevertheless, independently of the specific data, the trends of the digestion kinetic constants obtained with tested starches by means of both

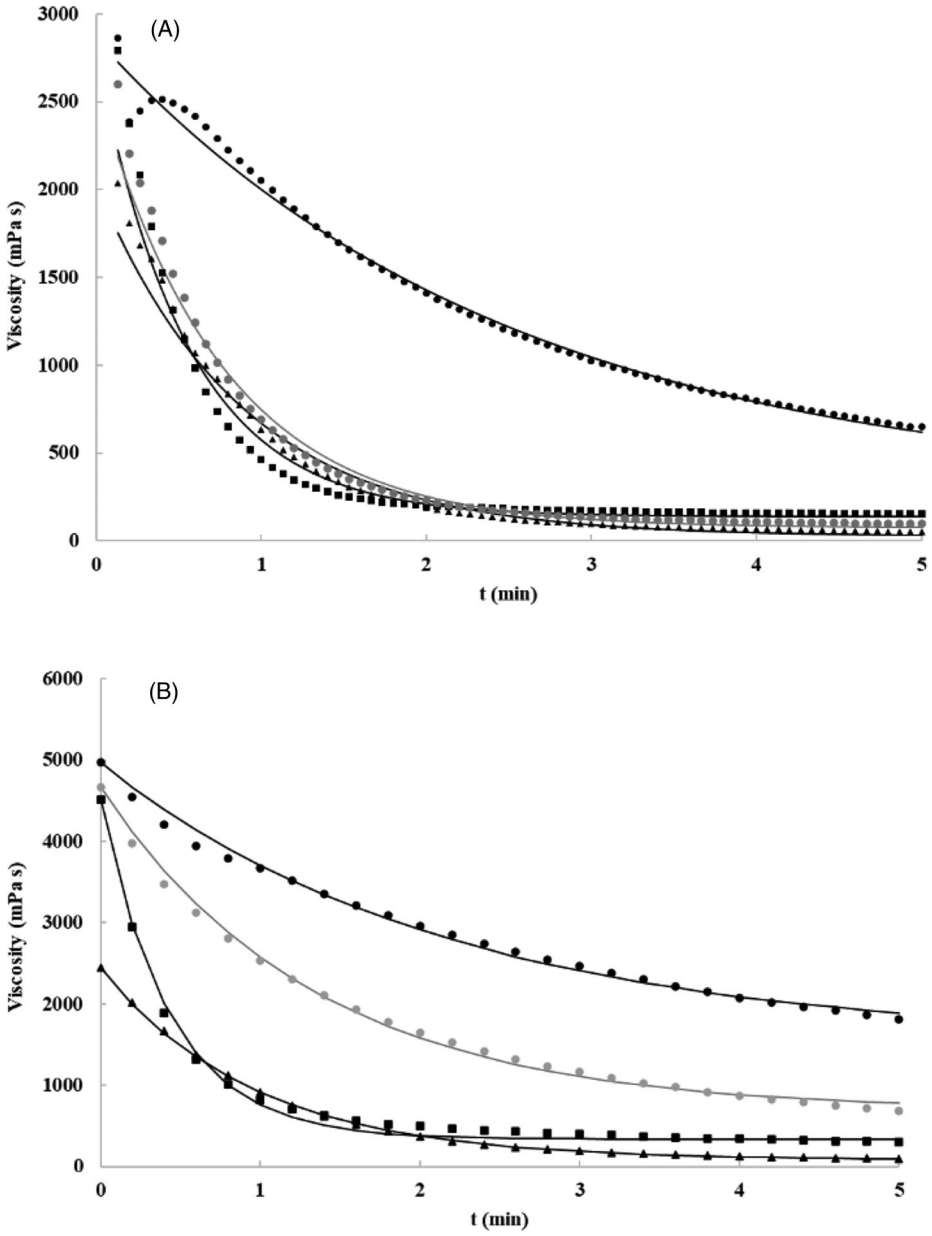

Figure 4. Variation of apparent viscosity during hydrolysis of corn (●), corn pH 5.8 (●), wheat (■), and rice (▲) starchy gels and their modeling by Equation (1) (▬). A) RVA digestograms and B) Rheometer digestograms.

methods (RVA and rheology) were satisfactorily in agreement. Regarding $\mu_{\infty,}$ the lowest value was determined for rice starch (83 mPa s) and the highest with corn (1549 mPa s). Results confirmed the viability of those test to follow enzymatic hydrolysis simulating digestion, being able to discriminate among the type of starches. Conversely, the quantification of glucose release did not show significant differences in their hydrolysis rate.

2.4. Normalized Digestograms

Digestograms were the results of a decrease in viscosity due to the enzymatic hydrolysis of gelatinized starch. To visualize jointly the hydrolysis kinetics of tested starchy gels, **Figure 5** shows the corresponding normalized curves (μN vs dimensionless time, t/t_{final}) of hydrolysis kinetics. Sorba et al.[9] made similar adjustment for studying retrograded gels. Normalized viscosity μN (–) was evaluated considering μ_0 and μ_∞ values by Equation (2), against the results of the biochemical kinetic (C/C_0) in reference to glucose content.

$$\mu_N = \frac{\mu_t - \mu_\infty}{\mu_0 - \mu_\infty} \tag{2}$$

Regardless of the botanical origin of the starch, it can be observed the sharp drop of μN for wheat, intermediate one for rice,

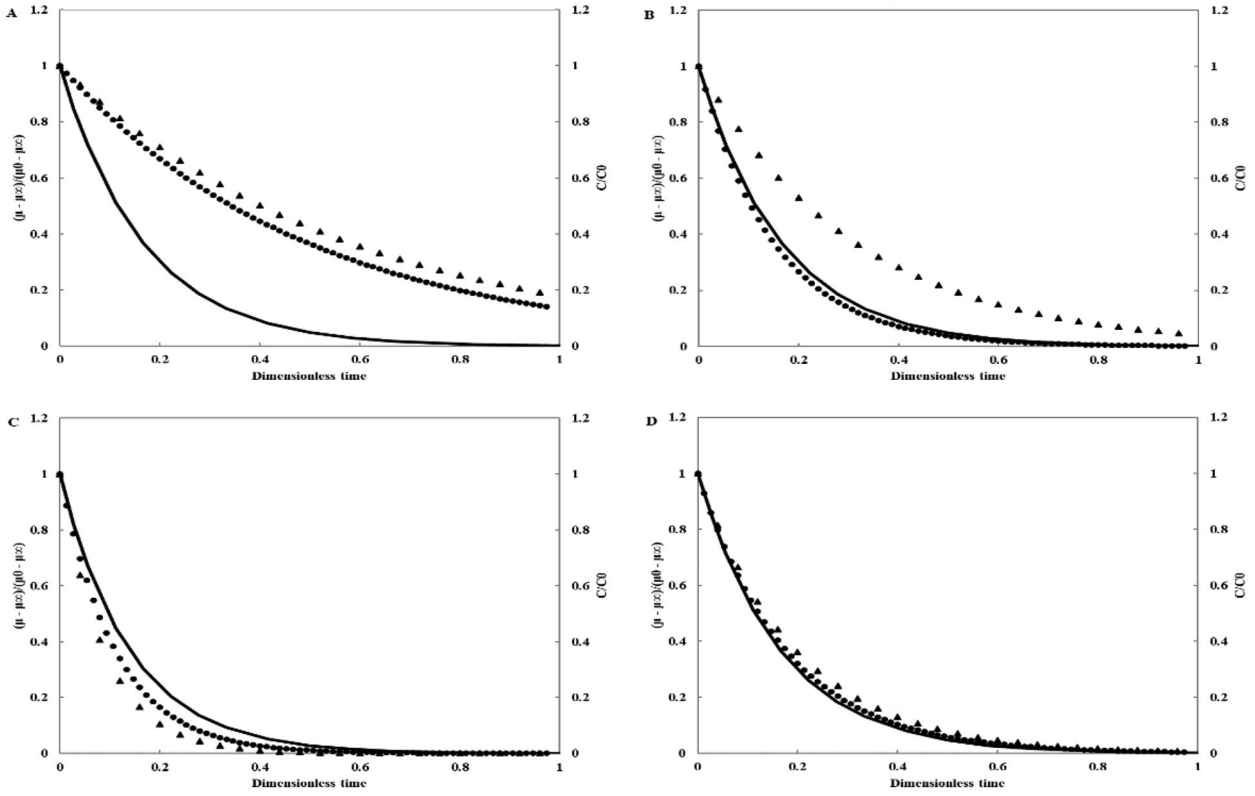

Figure 5. Normalized curve of apparent viscosity using Equation (2) during different hydrolysis: biochemical (▬) RVA (●) and rheometer (▲) methods. Corn A), corn pH 5.8 B), wheat C), and rice D). Biochemical hydrolysis time on the lower X-axis and digestograms time on the upper X-axis.

and moderate drop for corn starch gels (Figure 5). These curves showed the differences in the hydrolysis time of digestible starch in the gels. Then, all curves were asymptotic at long times (all digestible starch was already hydrolyzed). Corn starch was the exception, but it was confirmed that the pH of the sample was a factor that modifies the rheological behavior, mainly in the RVA method. This indicated that the analysis had to be carried out at an optimal pH for the enzymatic activity. In the case of biochemical hydrolysis, the pH of the corn starch gel did not vary the normalized viscosity plots, that was expected since gels pH effect is negligible when diluted into the buffer solution. The models used allowed to know the rate of starch digestion (Table 1), having very good fitting RVA ($R^2 > 0.99$), rheometer ($R^2 > 0.99$), and biochemical kinetics ($R^2 > 0.96$). Differences in the fitting might be attributed to the recording time in each methodology, RVA and rheometer quantifies the viscosity every 4 and 12 s, respectively, whereas aliquots for the biochemical analysis were withdrawn every 5, 15, or 30 min along the enzymatic assay. Most of the starch is digested, at relative high rate, for short period of time when following the apparent viscosity. In both methodologies, wheat gel showed higher hydrolysis rate (k), which indicated that the digestion was faster compared to other starches.

3. Conclusions

Single tests were developed to study the gelatinization performance and the digestion of different starch gels. Viscosity changes of different starches recorded with RVA or rheometer followed by amylase hydrolysis provide digestograms that were used to predict gels digestion by fitting experimental results to a first-order kinetic models. Parameters obtained from the fitting can be used for predicting starch digestion using rapid, simple, and reliable methods. Those can be used to carry out preliminary studies of many samples and identify the rheological behavior with alpha-amylase addition. A preliminary discrimination for predicting starch behavior might be very useful prior to in vitro or in vivo digestions.

4. Experimental Section

Materials: Starches from corn and wheat (EPSA, Valencia, Spain) and rice (Sigma Aldrich, Sigma Chemical, St. Louis, MO, USA) were employed. Moisture content of the starches was 13.08%, 12.60%, and 10.56%, for corn, wheat, and rice, respectively. The enzymes used were VI-B α-amylase from porcine pancreas (EC 3.2.1.1) from Sigma Aldrich (Sigma Chemical, St. Louis, MO, USA) and amyloglucosidase (EC 3.2.1.3) provided by Novozymes (Bagsvaerd, Denmark). Glucose oxidase/peroxidase (GOPOD) kit (Megazyme International Ireland Ltd., Bray, Ireland) was used. All reagents were of analytical grade. Solutions and standards were prepared using deionized water.

Change in Viscosity of Gel and its Hydrolysis Using the Rapid Visco Analyzer: Three grams (14% moisture basis) of starch were placed into the RVA canister and dispersed in 25 mL distilled water. The pH of slurries was determined. Tests were performed in the Rapid Visco Analyzer (RVA 4500; Perten Instruments, Hägersten, Sweden) using the following settings: 50 °C for 1 min, heating from 50 to 95 °C at 10 °C min^{-1}, holding at 95 °C for 2.5 min, cooling down to 37 °C at 10 °C min^{-1}, followed by holding at 37 °C for 36 s for adding the α-amylase solution (900 U mL^{-1}

solution), and then continue recording viscosity at 37 °C for 5 min. Preliminary assays were conducted with corn starch to select the amount of α-amylase (Figure S1, Supporting Information). Different concentrations of α-amylase (56, 90, 169, 225 U) were tested and the enzyme content that induced an intermediate hydrolysis rate was selected (90 U 100 μL^{-1} solution that represented 30 U g^{-1} of starch). Temperature within the slurry/gel was recorded using a Comark N2014 multi-sensor temperature data logger (Comark Instruments, Norwich, Norfolk, UK). Temperature readings were recorded every second. Rotational speed in the first 10 s was 960 rpm and then it was kept at 160 rpm along the test, except when the protocol was stopped (0 rpm) for enzyme addition. Apparent viscosity (mPa s) of starches without adding enzyme was also recorded as reference. RVA analysis was carried out at least duplicate. Pasting parameters extracted from the recorded data included: onset time (min), at which starch viscosity started to increase during heating, peak viscosity (maximum viscosity during heating), peak time (min, at which maximum viscosity is reached), trough viscosity (minimum viscosity when holding at 95 °C), breakdown (difference between maximum and trough viscosity), setback (difference between viscosity at 37 °C and trough viscosity), initial (after adding the enzyme), and final (at the end of the assay) viscosity during the enzymatic hydrolysis.

Rheology of Starch Gels and Enzymatic Hydrolysis Using a Rheometer: The rheological experiments were carried out with a stress-controlled rheometer (MCR 301; Anton Paar Physica, Graz, Austria) using a starch pasting cell (ST24-2D/2V/2V-30) with the following settings: measuring bob radius of 12.00 mm, cup radius of 14.46 mm, and a gap of 2.46 mm. A solvent trap kit was used to minimize water evaporation during tests. A similar protocol, regarding starch concentration (3 g—14% moisture basis—in 25 mL distilled water), times, and temperatures, to the one described above for the RVA, was defined to monitor in the rheometer the gel formation followed by the starch hydrolysis. A pre-shear at 100 rad s^{-1} (960 rpm), 50 °C for 10 s was applied to achieve sample homogenization, followed by a holding time for 1 min at 50 °C and 18 rad s^{-1} (160 rpm). This shear rate was kept for the rest of the assay. A temperature sweep was carried out from 50 to 95 °C at 10 °C min^{-1} to form the gel. High temperature of 95 °C was maintained for 2.5 min. Then, a temperature sweep was made from 95 to 37 °C at 5 °C min^{-1} to achieve the required temperature to make the enzymatic hydrolysis. A rest time of 36 s was needed to introduce the α-amylase (as described in RVA section). Finally, apparent viscosity, μ, at 37 °C for 5 min was monitored to assess the evolution during starch hydrolysis.

Starch Gels Digestion by In Vitro Enzymatic Method: Gels from different starches were prepared in the RVA using Standard 1 method provided by supplier. Starch gels were subjected to hydrolysis digestion following the method reported.[21] Experimental hydrolysis data were used to calculate rapidly digestible starch (RDS) or fraction hydrolyzed during the first 20 min, and the slowly digestible starch (SDS) hydrolyzed within 20 and 120 min.[24] Data were also fitted to a first-order Equation (3) to obtain the kinetic parameters of gels hydrolysis[25]:

$$C = C_{\infty}\ \left(1 - e^{-kt}\right) \tag{3}$$

where C was the concentration (g/100 g gel) of starch hydrolyzed at t time (min), C_{∞} (g/ 100 g gel) was the maximum hydrolysis of starch gels, k (min^{-1}) was the kinetic constant and t was the selected time.

Statistical Data Analysis: The Microsoft Excel Solver was used to model first-order kinetic equations. The digestion results obtained by different methodologies were correlated using Statgraphics Centurion XVII software (Statistical Graphics Corporation, Rockville, MD, USA) by means of analysis of variance (ANOVA) with Fisher's least significant differences test (LSD). Experimental data were expressed as mean ± standard deviation and $p < 0.05$ were considered significant.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

M.S.: conceptualization; data curation; formal analysis; investigation; methodology; roles/writing – original draft; L.M.: investigation; methodology; R.G.: methodology; supervision; data curation; R.M.: formal analysis; writing – review & editing; funding acquisition; C.M.R.: conceptualization; funding acquisition; investigation; supervision; writing – review & editing.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

in vitro digestion, kinetics, modeling, rapid visco analyzers, rheometers

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