Food Biophysics (2010) 5:149–160
DOI 10.1007/s11483-010-9155-2

ORIGINAL ARTICLE

Influence of Guar Gum on the In Vitro Starch Digestibility—Rheological and Microstructural Characteristics

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Published online: 30 March 2010

Abstract The present study investigates the effect of guar gum on the digestibility of a waxy maize starch in vitro under simulated gastric and intestinal conditions. A detailed rheology and confocal scanning laser microscopy of the digesta were performed in order to study the possible mechanisms of interactions involved during in vitro hydrolysis of starch. No starch hydrolysis was observed under simulated gastric conditions, whereas more than 90% hydrolysis was observed at the end of digestion under simulated intestinal conditions. In the presence of guar gum, the starch hydrolysis was reduced by nearly 25% during the first 10 min and by 15% at the end of in vitro intestinal digestion. The rheological behavior of the digesta was significantly affected in the presence of the gum. The viscosity of digesta decreased during intestinal digestion; however, the extent of decrease was quite low in the presence of guar gum. The consistency index increased and flow behavior index of digesta decreased in the presence of gum after 1 min of intestinal digestion. All the samples (digested or undigested) displayed a pseudoplastic behavior as their apparent viscosity (η_a) decreased with an increase in shear rate. A negative correlation between the starch hydrolysis (%) and storage modulus for the starch sample without gum and a positive correlation for the starch sample with gum were found. Large granule remnants observed through confocal micrographs have shown that the solubilization of starch granule remnants during in vitro digestion is significantly reduced in the presence of gum.

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Keywords Starch · Guar gum · In vitro starch hydrolysis · Rheology · Confocal laser scanning microscopy · Particle size analysis

Introduction

Dietary carbohydrates are digested and absorbed at different rates and to different extents in the human small intestine depending on their botanical source and the physical form of the food.[1] Therefore, the levels of glucose and insulin in circulating blood following a meal (the postprandial blood glucose and insulin responses) differ with different foods. Starch is the largest source of carbohydrates in the human food, and it can be classified into rapidly digestible, slowly digestible (SDS), or resistant starch depending on its rate of digestibility.[2] The diets containing large amounts of rapidly digestible starch raise the blood glucose and insulin responses in the body.[1,3] A lower or attenuated response is considered to be beneficial to both healthy and diabetic people, and the diets rich in slowly digestible carbohydrates may help protect against chronic diseases.

There is a steady rise in the use of plant-derived polysaccharides (gums) in different food products because of the rapid increase in the consumption of ready-made meals and novelty foods and also because of the consumers' growing awareness of the need to increase the amount of fiber and reduce the amount of fat in the diet.[4] Guar gum is a galactomannan which is generally used in starch-based products as a thickening, emulsifying, and stabilizing agent. It is a good source of dietary fiber and contains 80% total dietary fiber in the soluble form.[5,6] Soluble dietary fiber acts like a sponge and absorbs water in the intestine, mixes with the food to form an entangled

network, and thereby slows down the rate of digestion and absorption.[5,7] The effects of gums on the human metabolism are considered to be beneficial because they have been reported to decrease postprandial glycemia following ingestion of starchy food.[8] Most of their actions in the upper gut could be related to their ability to produce high viscosity (at low concentration) in the gut lumen, thereby significantly affecting the nutrient absorption and postprandial plasma nutrient levels.[7,9–11] The other beneficial effects such as the production of short-chain fatty acids are related to the fermentation of gums by microflora in the large intestine.[12] However, bacterial fermentation of viscous polysaccharides has several clinical implications like gas production which may result in distension and flatulence.[13–15]

Controlling the postprandial rise in blood glucose after ingestion of starchy food is of importance in the management of diabetes. The galactomannan seed gums such as guar gum are considered as functional ingredients for lowering blood cholesterol, postprandial blood glucose, and insulin levels. The aim of this study was to investigate the effect of guar gum on the digestibility of a highly digestible, waxy maize starch in vitro. Rheology and confocal scanning laser microscopy were also performed in order to study the structural organization of the guar gum and starch matrix.

Materials and Methods

Materials

Native waxy (Amioca, ~99% amylopectin), normal maize (Avon, ~25% amylose) starches, and commercial guar gum (Hicol U) used in the study were procured from National Starch and Chemical NZ Ltd. (Auckland), Penford New Zealand Ltd. (Auckland), and Chemiplas NZ Ltd. (Auckland), respectively. Pepsin (porcine gastric mucosa, 800–2,500 U/mg protein), pancreatin (hog pancreas, 4× USP), and invertase (Invertase, Grade VII from bakers yeast, 401 U/mg solid) were purchased from Sigma-Aldrich Ltd. (St Louis, USA). Amyloglucosidase (3,260 U/mL) was supplied by Megazyme International Ireland Ltd. (Ireland). All the other chemicals used were of analytical grade.

Methods

Sample Preparation

Waxy maize starch and guar gum powders were mixed and then dissolved in water (reverse osmosis purified water) and cooked at 100 °C for 20 min to completely gelatinize the starch. To obtain a starch–gum mixture which can be mixed uniformly in the digestion reactor, trials were performed using different concentrations of the starch and gum mixtures, e.g., 3% (*w*/*w*) and 4% (*w*/*w*) starch containing 0% to 1.5% guar gum and their mixing ability judged visually. Four percent starch and 1% gum was chosen as the best mixture for further experimentation.

In Vitro Digestibility of Starch

A two-stage model system to represent gastric and intestinal digestion of starches was used. The simulated gastric (SGF) and intestinal (SIF) fluids were prepared in accordance with the US Pharmacopeia.[16] One hundred seventy grams of cooked and cooled starch or starch–gum mixture (prepared as explained in the previous section) was added to a jacketed glass reactor (500-mL capacity) and stirred mechanically at 300 rpm. The reactor jacket was connected to a circulatory water bath to maintain its temperature at 37±1 °C. The pH was adjusted to 1.2 and SGF (30 mL) containing pepsin (enzyme/starch (dry weight basis) ratio, 1.765:100, *w*/*w*) was then added to start the hydrolysis. After 30 min, pepsin was inactivated by changing the pH to 6.8 using 1 M NaOH. During digestion, pH was maintained at 1.2±0.1 by automatic titration with 0.5 M NaOH using a pH controller attached to a peristaltic pump. Aliquots were taken after 0 (control), 15, and 30 min of digestion and then immediately analyzed for reducing sugars (as described in the next section).

To perform the second step of digestion, SIF (35.3 mL) containing pancreatin (enzyme/starch (dry weight basis) ratio, 1.3:100, *w*/*w*), amyloglucosidase (enzyme/starch (dry weight basis) ratio, 0.26:1, *v*/*w*), and invertase (enzyme/starch (dry weight basis) ratio, 1:1,000, *w*/*w*) was added to the reaction mixture to simulate digestion in the small intestine. Mixtures were maintained at 37 °C and gently stirred (300 rpm) for a further 2 h and the pH maintained at 6.8±0.1 throughout this step. Aliquots (0.5 mL) were withdrawn at 31, 35, 40, 45, 60, 75, 90, 105, 120, 135, and 150 min of digestion and then immediately analyzed for reducing sugars.

Analysis of Reducing Sugars

The samples taken at different digestion times were mixed with 2 mL of absolute ethanol in order to stop the enzymatic hydrolysis. After leaving the solution for 30 min, 0.1 mL of the ethanolic solution was incubated for 10 min at 37 °C with amyloglucosidase/invertase in acetate buffer (4 mg invertase, 0.1 mL amyloglucosidase per 10 mL acetate buffer, pH 5.2).[17] This was done to convert all the small sugars produced during hydrolysis to glucose which was analyzed using 3,5-dinitrosalicylic acid

method. The results were expressed as percent starch hydrolysis using the following equations:

$$\% S_H = S_h/S_i \quad (1)$$

$$= 0.9 \times G_p/S_i \quad (2)$$

where % S_H is the percent starch hydrolysis (total), S_h the amount of starch hydrolyzed, S_i the initial amount of starch (g), and G_p the amount of glucose produced (g). A conversion factor (from starch to glucose) of 0.9 which is generally calculated from the molecular weight of starch monomer/molecular weight of glucose (162/180=0.9) was used.[18,19]

Rheology

General

Small-amplitude oscillatory rheological measurements were carried out on the starch or starch–gum pastes and their digests with a dynamic rheometer (Physica MCR 301, Anton Paar GmbH, Germany) equipped with a cup and bob measuring system (DG 26-7 and C-PTD 200) or a cone and plate geometry (CP 40-4 and P-PTD200/56, gap = 0.049 μm). As required, strain sweep tests were first performed for all samples to determine the linear viscoelastic region. Starch or starch–gum pastes and digests were prepared for rheological experimentation as described in "In Vitro Digestibility of starch." Because no hydrolysis was observed under simulated gastric conditions, in vitro digestion was performed on the cooked and cooled sample pastes under simulated intestinal conditions only. In order to avoid the diluting effect of the simulated intestinal juice, the volume of juice added was reduced to one third while keeping the same enzyme activity per gram starch (three times concentrated juice). Samples were withdrawn from the digestion reactor for rheological experimentation after 30 s, 1 min, and 5 min of hydrolysis and their pH immediately changed to ~ 2 using HCl to stop the enzymatic reaction. After 5 min of hydrolysis, the viscosity of some samples was too low to perform rheological measurements. Control starch or gum samples were prepared in the same way except no enzymes were added. All the rheological measurements were performed in triplicate.

Steady Shear Tests

Viscosity flow curves were first obtained using the cup and bob measuring system at 20±0.1 °C with the operating shear rate ranging from 0.1 to 1,000 s^{-1}. Consistency index and flow index were calculated for all the samples using the power law model or Ostwald–De Waele law:

$$\eta_a(\dot{\gamma}) = C\dot{\gamma}^{F-1} \quad (3)$$

where η_a is the apparent viscosity, $\dot{\gamma}$ is the shear rate, C is the coefficient of flow or consistency index, and F the flow behavior index. Linear regression analysis was applied to the data for each sample in order to calculate flow behavior index and the consistency coefficient.

Frequency Sweep Tests

Small-amplitude oscillatory rheological measurements were performed on the digested and undigested starch–gum samples using a cup and bob measuring system. Strain sweep tests were first performed on the samples at a frequency of 0.1 Hz to determine the linear viscoelastic region. The frequency sweep tests were carried out from 0.1–10 Hz at a constant strain of 1% and temperature of 20 °C.

Time Sweep Tests

Time sweep experiments were performed using a cone and plate geometry to monitor the effect of starch or starch–gum paste hydrolysis on the rheological parameters online. Cooked sample paste (prepared as described in sample preparation) was added to the rheometer plate at 4 °C. The enzymatic solution (simulated intestinal juice) was added (starch/enzyme ratio kept the same as described in "General") and mixed thoroughly using a pipette tip. During the first step, the temperature was raised from 4 °C to 37 °C (in ~1 min) to initiate hydrolysis, and then the test was performed for 50 min at 37 °C at a strain and frequency of 1% and 1 Hz, respectively.

Confocal Laser Scanning Microscopy

The samples of starch (4%, *w*/*w*) or starch–gum dispersions (4% starch and 1% gum, *w*/*w*) were prepared as described in "In Vitro Digestibility of Starch." The normal maize starch samples were also digested under simulated intestinal conditions for comparison purpose. The samples of the digesta were taken at different intervals during intestinal digestion. Rhodamine B was used as the dye for the non-covalent staining of starch. The dye was mixed with the pastes or digesta as described previously.[20] A drop of the stained paste was placed on a glass slide; a coverslip was applied and then examined with a Leica model TCS SP5 DM6000B confocal laser scanning microscope (Leica Microsystems, Wetzlar, Germany).

Particle Size Analysis

The particle size diameter distributions of undigested starch–gum and starch only pastes were determined at room temperature with a laser diffraction particle size analyzer (Mastersizer Scirocco 2000, Malvern Instruments Limited, UK). The starch or starch–gum pastes were prepared as described in the sample preparation ("Methods"). Some portion of the paste was loaded into the small volume sample presentation unit of the Mastersizer to obtain an obscuration level of 15%. Refractive indices of 1.530 and 1.330 were used for the starch and liquid phases, respectively, while the starch granule absorption was set at 0.1.

Statistical Analysis

The data reported are averages of triplicate observations. The data were subjected to analysis of variance and Tukey's test to assess the significance of differences ($p<0.05$). The Pearson correlation coefficients for the relationship between different parameters were calculated using Minitab Statistical Software version 15 (Minitab Inc., USA).

Results and Discussion

In Vitro Digestion

Hydrolysis (%) of waxy maize starch during in vitro digestion in the presence or absence of guar gum is presented in Fig. 1a. No hydrolysis of starch occurred under the simulated gastric conditions (first 30 min) because of the absence of starch-hydrolyzing enzymes. However, when the SIF was added to the reaction mixture, the starch was rapidly digested by the pancreatic amylases. Approximately 70% of the starch (with no gum) was digested within the first 10 min of simulated intestinal digestion. This result was expected as waxy maize starch is considered to be highly and rapidly digestible among the starches and contains mainly amylopectin, which is more susceptible toward amylolytic attack.[21] However, this percentage cannot be interpreted as the percentage of hydrolysis of starchy food in vivo as the latter is much more complex. Other food components like proteins or fatty acids have been shown to have inhibitory effect on the starch hydrolysis.[22–24]

Addition of guar gum in the starch matrix led to a significant decrease in both the rate and the extent of final starch hydrolysis, as observed from the slope of the curve (Fig. 1a). A drop of ~25% in the starch hydrolysis (to that of the control) was observed during the first 10 min of hydrolysis when guar gum was added. Also, guar gum affected the final hydrolysis of the starch significantly ($p<0.05$), with an approx. 15% drop in the hydrolysis at the end of the digestion period. The rate and extent of starch hydrolysis in the small intestine are dependent upon several intrinsic and extrinsic factors.[2] SDS containing foods have been reported to improve the postprandial response of individuals with type 2 diabetes and may also prolong satiety.[21] It has been reported that the decrease in postprandial glycemia by gums following ingestion of starchy food could be due to their ability to produce high viscosity in the gut lumen, thereby significantly affecting the nutrient absorption and postprandial plasma nutrient levels.[12] The slower rate of starch hydrolysis in the presence of guar gum may be attributed to the gum's capacity to increase the viscosity of the digesta due to enlargement of fully hydrated glactomannan chains.[25]

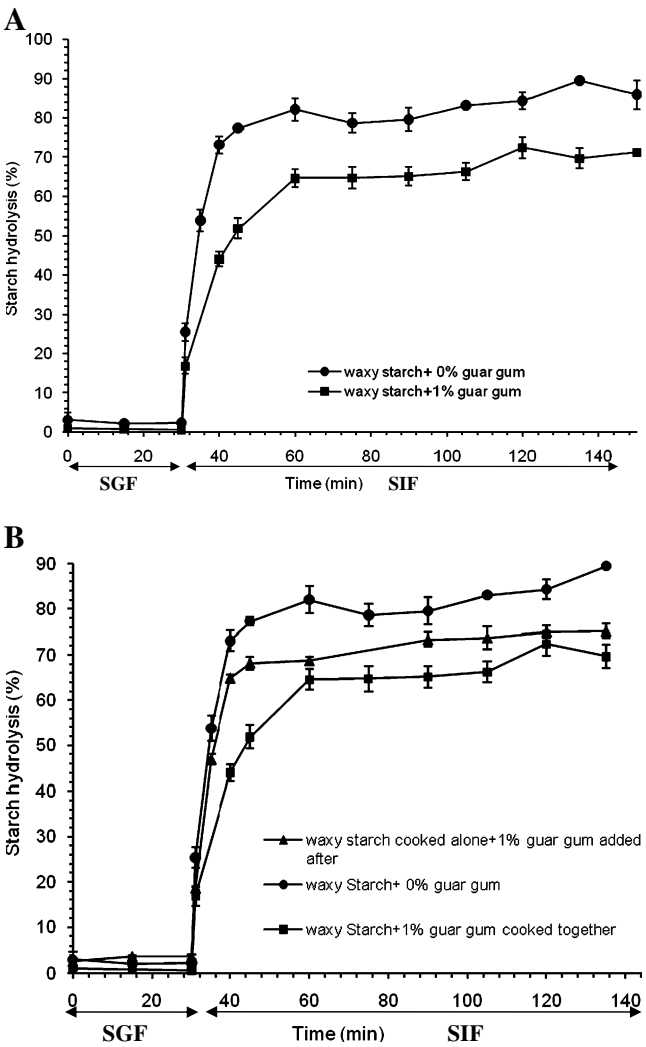

Fig. 1 **a** Effect of the addition of guar gum on the starch hydrolysis (%) during simulated gastric and intestinal digestion. **b** Effect of sample preparation method on starch hydrolysis (%) during simulated gastric and intestinal digestion

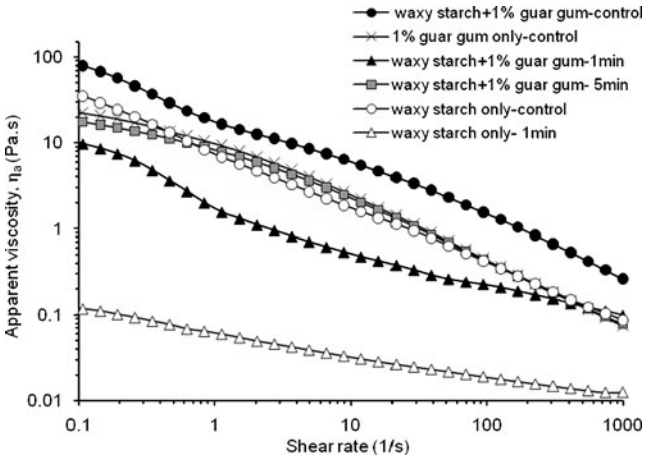

Fig. 2 Effect of digestion (30 s, 1 min) under simulated intestinal conditions on the flow behavior of the starch paste and guar gum samples

We also observed that guar gum increased the viscosity of the system significantly, which may have an effect not only on the mass transfer (diffusion coefficient) of the molecules (sugars and enzymes) but also on the enzymatic reactions. Hydrocolloids form a continuous network by suspending the starch granules in a coherent gel, which acts as a barrier when enzymes try to access the starch.[26] To check if viscosity is the only reason for the decrease in starch hydrolysis upon the addition of guar gum, the digestion period under simulated intestinal conditions was prolonged from 2 to 6 h. It was observed that no further starch hydrolysis took place between 2 and 6 h in the presence of gum (data not shown), which shows that the starch digestion is not only delayed but also reduced in the presence of gum. Therefore, the inhibition of the enzyme action permanently by the galactomannan molecules to prevent further hydrolysis of starch may not be ruled out.

The dry ingredients (starch and gum) were blended and then hydrated in accordance with the process generally followed in the industry. Assuming that this way of preparing the samples could have an influence on the water availability for starch gelatinization, starch was cooked separately and then mixed with the hydrated gum (keeping the dry ingredients/water ratio same as in the previously experiments) and then studied for the starch digestibility. Starch hydrolysis was observed to be somewhat faster; however, the hydrolysis was still lower than the starch alone (in the absence of gum; Fig. 1b). Limited water availability has an effect on the gelatinization and cooking of the starch,[27–29] so it could be concluded that as guar gum is highly hydrophilic, it may be acting partly by decreasing water availability. The restrictions imposed on the swelling of the starch granules by the galactomannan-based gums result in an alteration in the functional properties of starch gum pastes.[30] Song et al.[31] reported that the addition of hydrocolloids results in reduced swelling of starch granules because of the osmotic pressure generated within the continuous hydrocolloid phase. The effect of gum became more pronounced at higher concentration due to increased competition for free water molecules between gum and the starch granules.

Rheology

Flow Behavior

All the samples (digested or undigested) exhibited pseudoplastic behavior (Fig. 2). This is in agreement with the typical behavior of polymer solutions.[32] The addition of guar gum resulted in an increase in apparent viscosity of the starch paste (Fig. 2). The increase in viscosity can affect gastric function and may inhibit propulsive and mixing effects generated by peristalsis.[25] Under these conditions, interactions between substrates and digestives enzymes are less frequent. This may result in a decreased rate of starch digestion by α-amylase and ultimately a slower absorption of the hydrolysis products (e.g. maltose, α-limit dextrins). Solution viscosity has also been reported to influence the enzyme kinetics.[33]

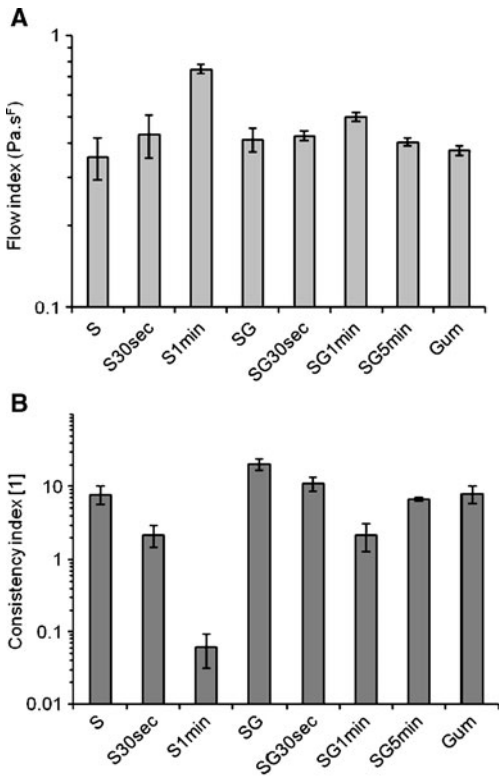

Fig. 3 **a** Effect of guar gum (*G*) on the flow behavior index during digestion (30 s, 1 and 5 min) under simulated intestinal conditions of starch (*S*) samples. **b** Effect of guar gum (*G*) on the consistency coefficient during digestion (30 s, 1 and 5 min) under simulated intestinal conditions of starch (*S*) samples

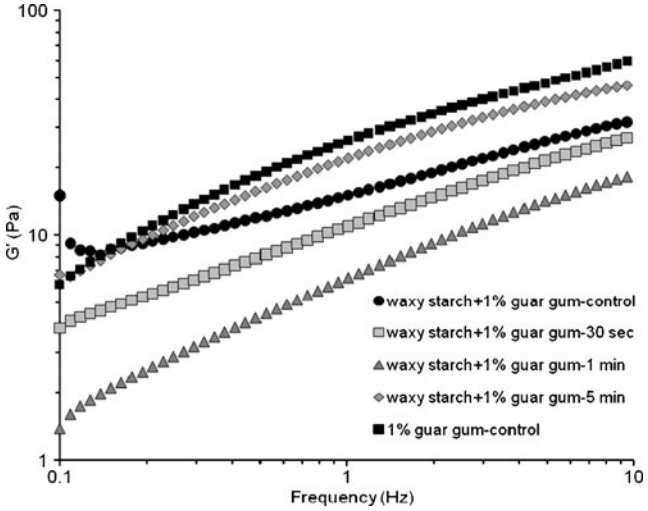

Fig. 4 Effect of digestion under simulated intestinal conditions (for 30 s, 1 min, and 5 min) on the storage (G') modulus of starch and/gum samples measured during frequency sweeps

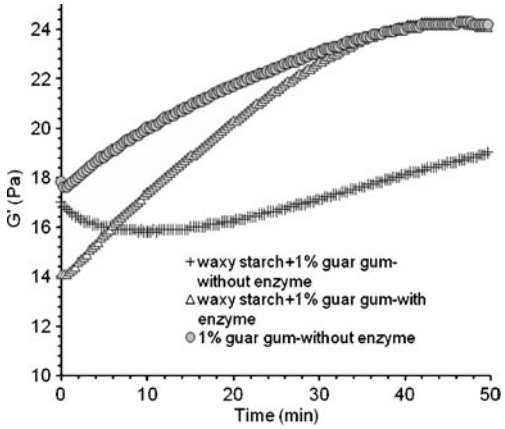

Fig. 5 Changes in dynamic rheological properties of starch and gum pastes in the presence or absence of enzymes at 37 °C and 1 Hz measured during time sweep experiment

As the digestion progressed, apparent viscosity was observed to decrease (for both with and without gum samples) compared with the control samples, which could be explained by the hydrolysis of the starch chains by pancreatic amylases (Fig. 2). However, for the starch and gum sample digested for 5 min, the apparent viscosity was greater than the sample digested for 1 min and was close to the gum only sample. The gum molecules may require sufficient time to fully hydrate by absorbing the water released during starch amylolysis. The 1-min duration may not seem enough for the gum molecules to balance the immediate viscosity loss that occurred during amylolytic attack, whereas it appears to be stabilized after the next 5 min. Similar observations were made for the consistency and the flow behavior indices presented in Fig. 3a, b. Hydrolysis resulted in an increase in the flow behavior index and a decrease in the consistency index.

Frequency Sweep Test

Mechanical spectra for G' and values of different rheological parameters from selected starch and gum systems before and after digestion under simulated intestinal conditions at 37 °C are presented in Fig. 4 and Table 1. A material whose G' and G'' are frequency-independent over a large timescale, with $G' > G''$, is generally solid-like. In contrast, strong frequency dependence suggests a material structure with molecular entanglements.[27] Such a material behaves more like a solid (lower tan δ) at higher frequencies and more like a liquid (higher tan δ) at lower frequencies.[34] For the control starch sample, both G' and G'' increased with increasing frequency at lower frequencies, with G' prevailing over G''. The digested starch sample (30 s) behaved in a similar manner but showed lower values for both G' and G'' than the control undigested starch sample. As the digestion progressed, G'' became dominant over G'. For the starch sample digested for 1 min, G'' was dominant over G' throughout the frequency range and no crossover frequencies were observed. The starch sample after 5 min of hydrolysis was not suitable for this test as the hydrolysis was nearly finished and its viscosity was close to the viscosity of water (10^{-2} Pa s). For the starch only samples, tan δ decreased in the order: starch digested for 1 min > starch digested for 30 s > starch control, which indicates that a solid-like starch behavior changes to a weakly structured fluid-like behavior upon digestion.

Table 1 Effect of digestion under simulated intestinal conditions (for 30 s, 1 min, and 5 min) on the storage (G'), loss moduli (G''), and loss factor (tan δ) of starch samples measured during frequency sweeps

Sample name	G' (Pa)	G'' (Pa)	tan δ
Waxy starch + 0% guar gum—control	7.05±1.61	2.66±0.65	0.38±0.03
Waxy starch + 0% guar gum—30 s	1.04±0.23	1.04±0.21	1.00±0.11
Waxy starch + 0% guar gum—1 min	0.10±0.03	0.21±0.06	2.08±0.13
1% guar gum—control	24.73±2.21	16.30±1.40	0.66±0.00
Waxy starch+1% guar gum—control	16.13±0.99	11.00±0.53	0.68±0.01
Waxy starch+1% guar gum—30 s	14.23±1.35	10.24±0.58	0.72±0.04
Waxy starch+1% guar gum—1 min	6.71±0.90	6.58±0.71	0.98±0.03
Waxy starch+1% guar gum—5 min	20.77±1.27	14.43±0.15	0.70±0.04

Values at 1-Hz frequency

Table 2 Changes in dynamic rheological properties of starch and gum pastes in the presence or absence of enzymes at 37 °C measured during time sweep experiment

Sample name	G' (Pa)				G'' (Pa)			
	0 min	5 min	30 min	50 min	0 min	5 min	30 min	50 min
Waxy starch + 0% guar gum—without enzyme	3.76±0.95	2.05±0.60	1.51±0.39	1.41±0.23	1.98±0.26	1.37±0.16	1.13±0.12	1.12±0.13
1% guar gum—without enzyme	31.45±1.48	17.35±1.05	21.60±1.12	21.20±1.24	18.8±0.99	14.65±0.34	16.2±0.41	15.75±0..33
Waxy starch+1% guar gum—without enzyme	20.87±1.25	12.05±1.53	13.34± 1.79	14.53± 1.27	14.37±0.46	8.80±0.55	9.19±0.38	9.15±0.49
Waxy starch + 0% guar gum—with enzyme	1.51±0.44	0.25±0.04	0.13±0.03	0.07±0.02	1.43±0.20	0.33±0.04	0.16±0.01	0.10±0.00
Waxy starch+1% guar gum—with enzyme	17.80±1.93	14.17±0.86	23.77±1.95	25.20±1.26	13.30±0.85	11.67±0.97	17.77±0.66	18.4±0.89

The addition of guar gum resulted in increased G' of the starch system. But both G' and G'' were lower than those of gum alone (Table 1 and Fig. 4). Guar gum inhibits the starch components from leaching out of the granules into the continuous phase of starch pastes during gelatinization, which results in an increase in the viscous characteristics.[35] For the digested starch and gum samples, it was observed that after 30 s and 1 min of digestion under simulated intestinal conditions, both G' and G'' were lower than for the control starch and gum sample. Surprisingly, for the sample digested for 1 min, the values for G' and G'' were almost the same and were lowest among all the starch–gum samples, whereas after 5 min of digestion, both these parameters were higher than the control and the sample's behavior during frequency sweep was close to that of the guar gum control sample.

No dramatic change in the rheological behavior during frequency sweeps was observed for the starch–gum samples upon digestion contrary to the starch only ones, which could mainly be attributed to the viscosity imparted by the guar galactomannan that stabilized the structure.

Time Sweep Tests

The rheological profiles and values of different rheological parameters from starch only and the starch–gum samples during hydrolysis, recorded by a time sweep experiment at 37 °C, are presented in Fig. 5 and Table 2. The profiles of the starch and starch–gum samples were observed to be considerably different, with the former showing very low values of the rheological parameters (G' and G'') during hydrolysis. The control starch sample showed a small drop

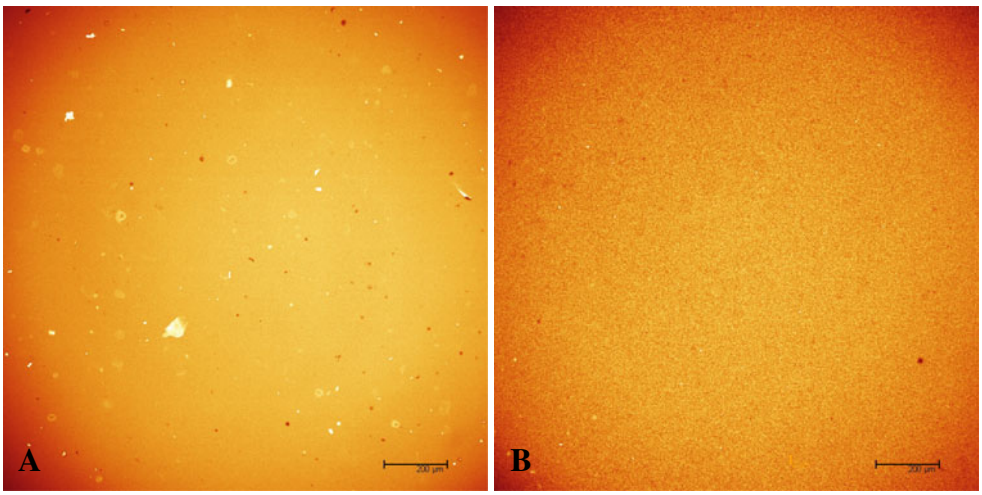

Fig. 6 Confocal micrographs showing the effect of digestion on waxy maize starch paste: waxy maize starch before digestion (**a**) and waxy maize starch after 5 min digestion under simulated intestinal conditions (**b**)

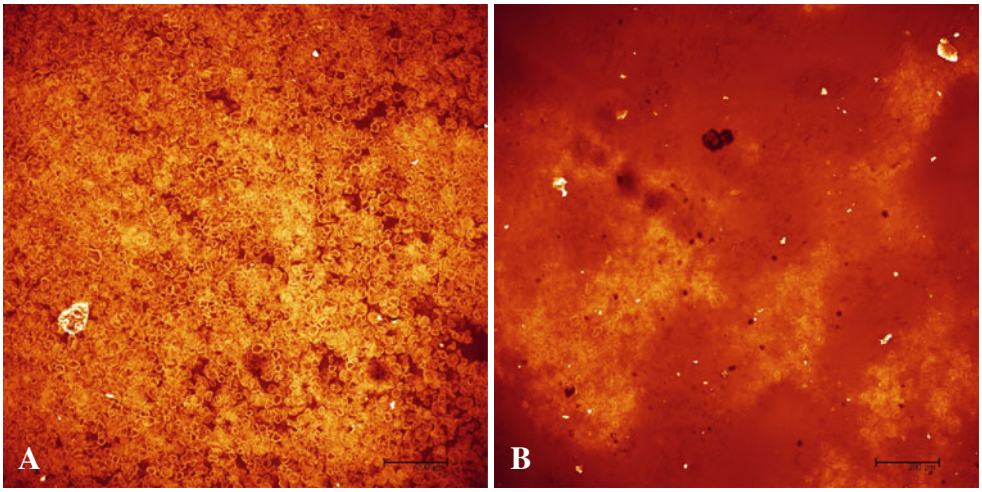

Fig. 7 Confocal micrographs showing the effect of digestion on normal maize starch paste: normal maize starch before digestion (**a**) and normal maize starch after 5 min of digestion under simulated intestinal conditions (**b**)

in both these parameters with time, especially G'. The addition of the enzymes led to hydrolysis of the starch chains, which resulted in a major drop in both G' and G'', which can be clearly observed for the starch only sample. The results of the time sweep are in accordance with the previous observations for starch–gum samples (Table 2 and Fig. 5). The starch–gum sample was observed to be stable during 50 min of the experiment, with negligible change in the storage or loss moduli. However, the addition of enzymes to the mixture led to an increase in both these parameters. After 30 min of digestion, the storage and loss moduli values and patterns of the starch–guar gum sample

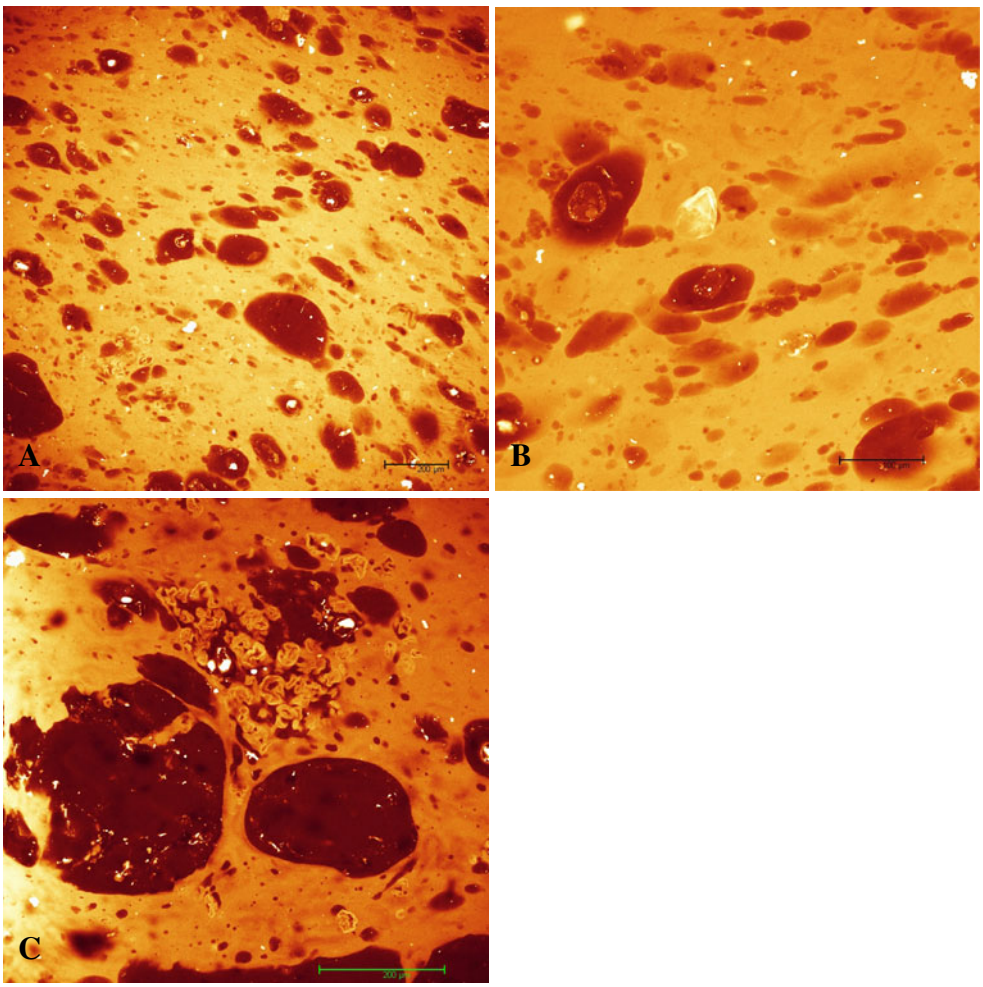

Fig. 8 Confocal micrographs showing the effect of the addition of guar gum in waxy maize starch paste at different magnifications

were almost similar to those of the guar gum control. Most of the starch in the starch–gum sample is hydrolyzed within the first 5 min. As the starch digestion progresses, water is liberated, allowing the gum present in the system to take up that water and swell further, which may have resulted in an increase in the G' and G''.

To check if there is a possible correlation between the amount of hydrolysis and the changes in the rheological parameters, Pearson correlation coefficients were calculated between the starch hydrolysis and the storage/loss moduli at different digestion times. Negative correlations between the starch hydrolysis (%) and both storage and loss moduli ($r=-0.961$; $r=-0.911$, $p<0.01$) for the starch sample without gum and positive correlation for the starch sample with gum($r=0.877$; $r=0.928$, $p<0.01$) were found. However, it should be pointed out that that the mixing conditions in the present system (i.e., cone and plate) are not comparable to the mixing conditions in the reactor or in the body.

The guar gum increases the viscosity of a food matrix at a relatively low polymer concentration and therefore seems to act by increasing the viscosity of the digesta in vitro. This may decrease the postprandial carbohydrate absorption after ingestion of the starchy food. Additionally, guar gum may influence the water availability in the matrix, which is important for the enzymatic activity. However, as the conditions in vivo are different in terms of shear, secretion, and mixing, some other factors/mechanisms may also play a role.

Confocal Laser Scanning Microscopy and Particle Size Analysis

Confocal laser scanning micrographs of the starch only and starch–gum samples are presented in Figs. 6, 7, 8, 9, and 10. As waxy starch is highly prone to cooking, most of the granules are dissolved during cooking and very few starch granule remnants are left after cooking for observation (Fig. 6a). Hydrolysis of the waxy starch by pancreatic amylases led to complete disappearance of the remnants after 5 min of digestion as shown by the homogeneous background (Fig. 6b).

To study the effect of guar gum clearly, normal maize starch was also used for confocal laser scanning microscopy along with the waxy maize starch. Normal maize starch

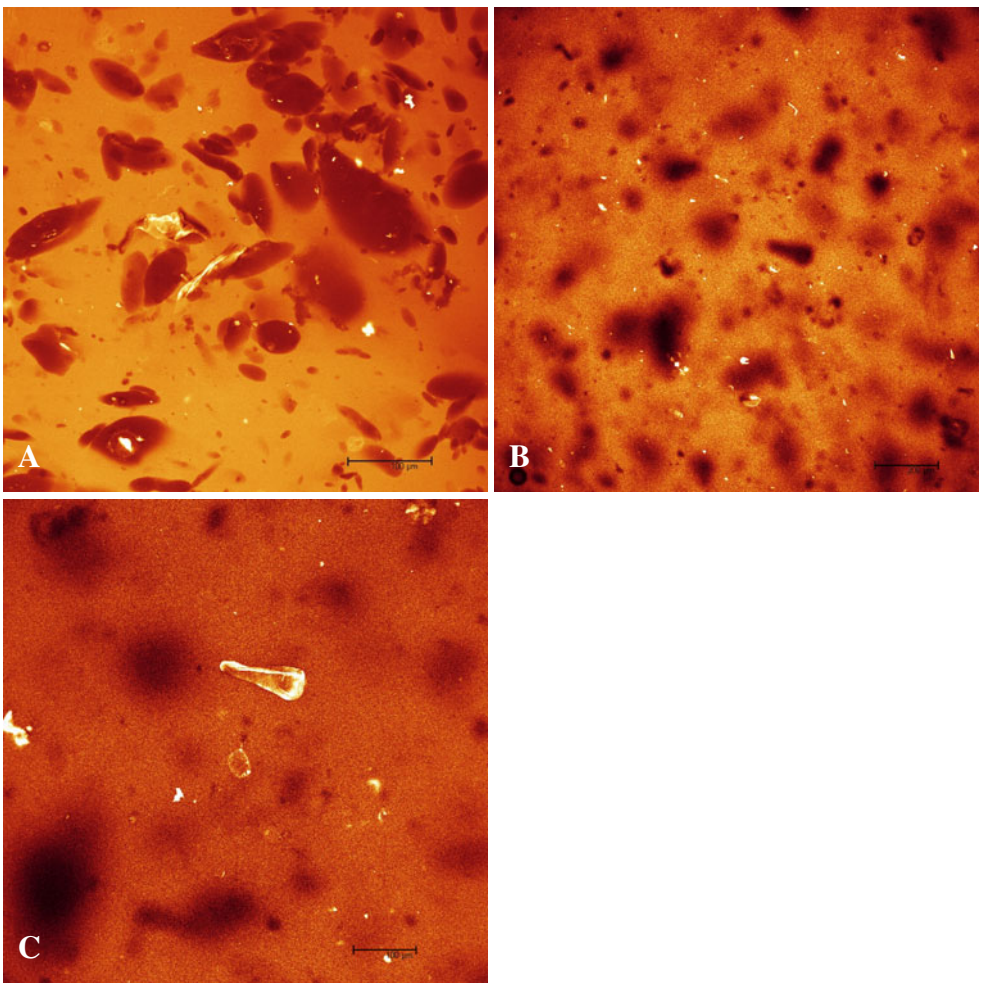

Fig. 9 Confocal micrographs showing the effect of digestion on waxy maize starch–gum paste: upon the addition of intestinal juice (0 min) before digestion (**a**), after 1 min (**b**), and after 5 min of digestion (**c**) under simulated intestinal conditions

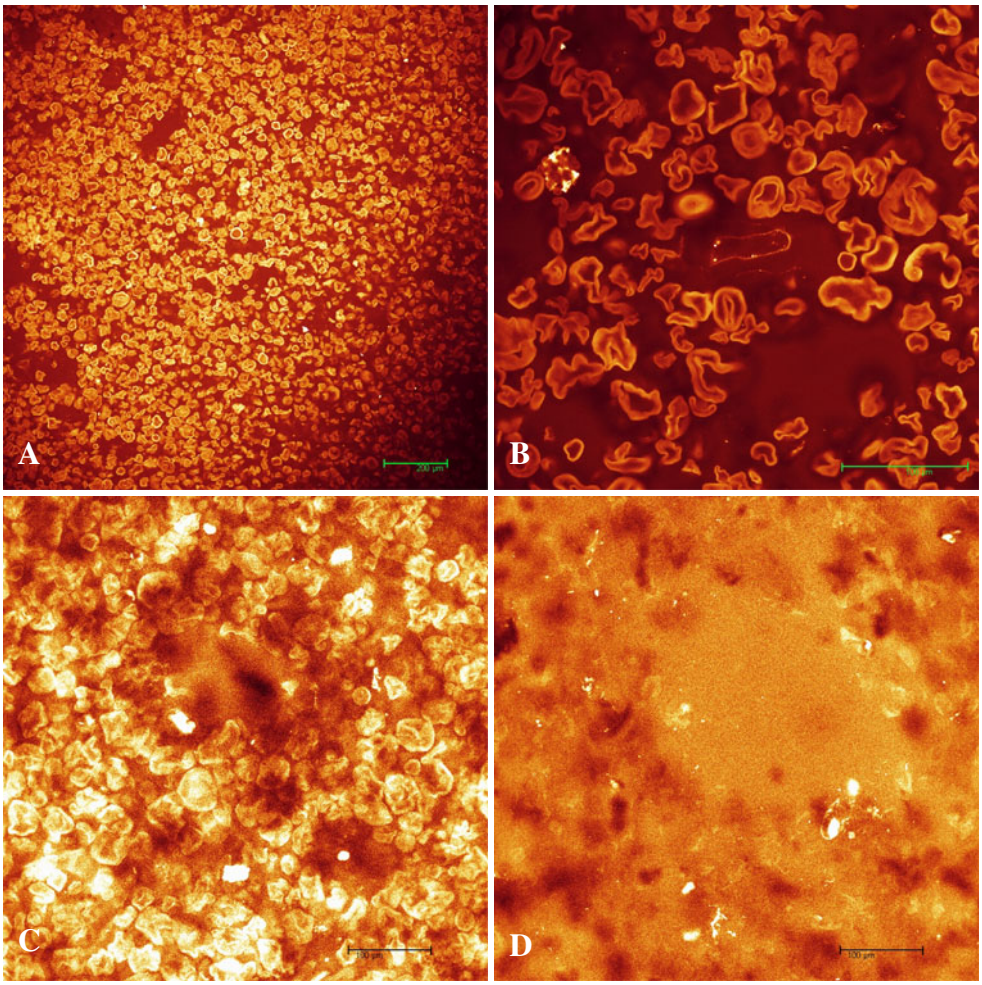

Fig. 10 Confocal micrographs showing the effect of digestion on normal maize starch–gum paste: upon the addition of intestinal juice (0 min) before digestion at different magnifications (**a**), after 1 min (**c**), and after 5 min of digestion (**c**) under simulated intestinal conditions

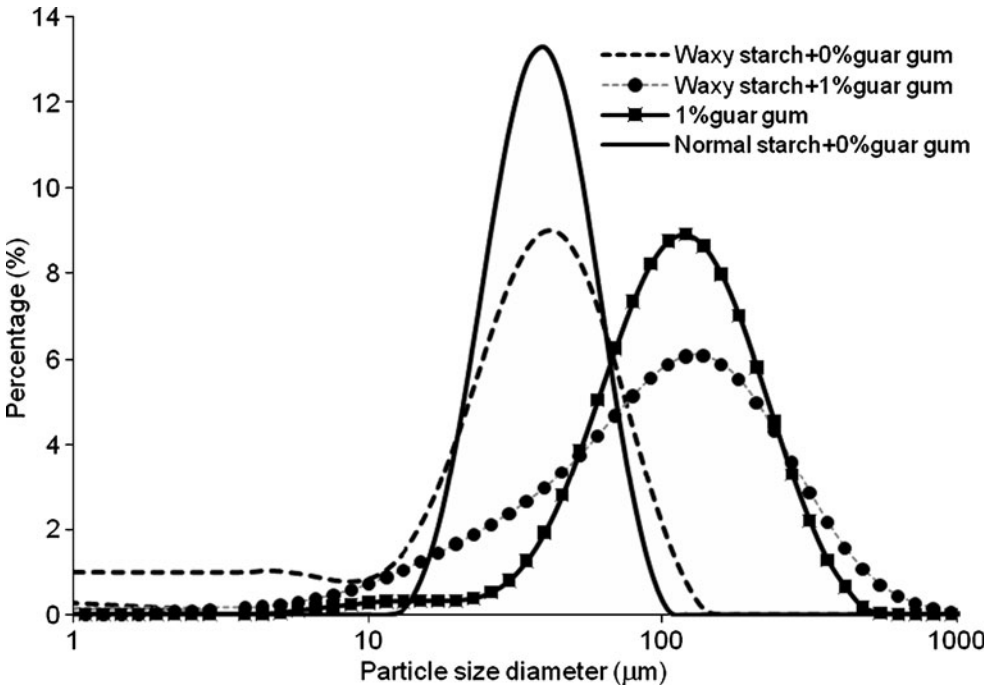

Fig. 11 Effect of guar gum (1%) on the particle size distribution of normal and waxy maize starch pastes

granules are more resistant toward cooking and showed numerous starch granule remnants in the starch paste (Fig. 7a). However, the in vitro digestion led to a decrease in the number of the remnants, showing progressive starch hydrolysis (Fig. 7b).

The addition of guar gum to both normal and waxy starches led to a delayed hydrolysis, as shown by the presence of undigested remnants after 5 min of hydrolysis (Figs. 8, 9, and 10). The gum layer around the starch granules (Fig. 8c) may have inhibited the access to the enzymes and, consequently, the enzymatic hydrolysis. Thus, guar galactomannan may function as a physical "barrier" to α-amylase/starch interaction and/or to the release of hydrolysis products into the aqueous phase of the digesta. The normal maize starch granule remnants in the presence of guar gum seemed larger in size compared with the starch sample without gum (Figs. 7a and 10a). Guar gum has been reported to decrease the abundance of the granule remnants or ghosts in the starchy paste by inhibiting the starch components from leaching out of the starch granule.[35] Starch ghosts are the gelatinized starch granule envelopes which are formed after the majority of internal starch polymers have been released.[36,37] Particle size analysis was performed to see the effect of gum on the particle size of the starch paste. The particle size diameter distribution for both waxy and normal maize starch pastes with and without the addition of guar gum is presented in Fig. 11. Guar gum contributed to an increase in the particles above the 50- to 100-μm diameter range, and a decrease was observed for the particles below this size range for waxy maize starch. As observed from the confocal microscopy images, many of the particles in the waxy starch–gum paste may be from the gum itself. For normal starch also, it was noticed that the distribution is moving to the right, which clearly shows the appearance of bigger particles in the presence of gum. This is in accordance with the microscopic observations.

Conclusions

Guar gum in the food matrix was observed to act not only by delaying but also by decreasing the extent of the starch hydrolysis. The gum may be acting by forming a barrier/layer around the granules, as observed using microscopy. This barrier may not only restrict the transfer of the enzymes to the granules but also change the starch granule swelling pattern during the gelatinization, resulting in a different granule remnant size distribution. Addition of guar gum to starch significantly increased the viscosity of the environment. It was observed that the factors linked to the mass transfer (consistency and flow behavior indices) were affected. The action of guar gum on the water availability in the matrix is also considerable, which may have an effect on the granule swelling and ultimately enzyme action during starch hydrolysis, as shown by the measurements of glucose release and microscopic investigations. The techniques used in the present study did not enable us to show a direct inhibition of the enzyme action by the galactomannan molecules, but further studies are in progress to investigate this. It has to be pointed out that in most of our experiments, the conditions were not exactly similar to the physiological conditions, so the results may not be interpreted in a quantitative way as several other factors also play a part in vivo.

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