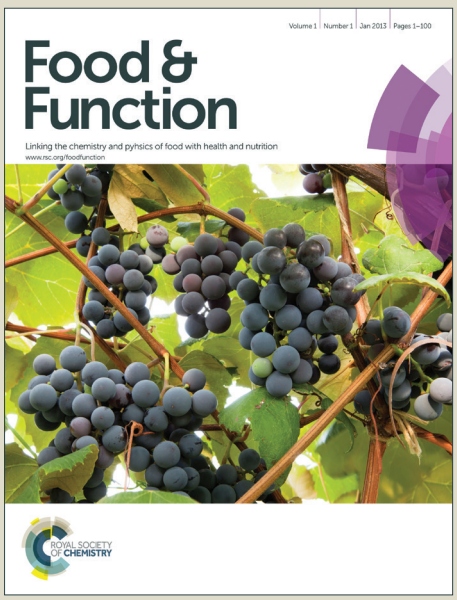
Food & Function

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/foodfunction

In vitro gastrointestinal digestibility of native, hydroxypropylated and cross-linked wheat starches

A.R. Yousefi[1,2], Seyed M.A. Razavi[2*], Abdolreza Norouzi[3]

[1]*Department of Polymer Engineering, University of Bonab, Iran*

[2]*Food Hydrocolloids Research Center, Department of Food Science and Technology, Ferdowsi University of Mashhad (FUM), PO Box 91775-1163, Mashhad, Iran*

[3]*Department of Nutrition Science, Medical University of Mashhad, Iran*

[*]Corresponding author. Tel.: +98 511 8795618; fax: +98 511 8787430. E-mail address: s.razavi@um.ac.ir

Abstract

The digestibility and estimated glycemic indices (GI) of native (NWS), cross-linked (CLWS) and hydroxypropylated wheat starches (HPWS) were obtained by *in vitro* enzymatic hydrolysis. The resistant starch (RS) content and GI were found to be 6.59 and 93.13 for NWS, 7.57 and 92.20 for CLWS, and also 13.15 and 89.04 for HPWS, respectively. The amounts of glucose release for CLWS were approximately 6-11%, and for HPWS were 16-19% lower than that for NWS after digestion at simulated intestinal condition (SIC). The linear and two-term exponential models were fitted well to the experimental glucose release data at simulated gastric condition (SGC) and SIC, respectively (R^2 = 0.858-0.991). After digestion at SIC, the consistency coefficient *(k)* values drastically decreased (73.02-90.27%), while the flow behavior index *(n)* increased (155.56-363.64%). Therefore, the amounts of glucose release can be controlled by manipulating the structure of native starches using chemical modifications such as cross-linking and hydroxypropylation.

Keywords: Chemical modification, Digestibility, Glucose release, Rheology, Starch

1. Introduction

Due to many nutritional, technological and textural advantages of starch in food products, it is receiving much more attention. Depending on the rate of digestibility, starches were classified into three categories consisting rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) [1]. Consuming starchy foods containing large amounts of RDS cause a rapid raise in blood glucose level which is followed by an increase in insulin response after ingestion [2-3]. Therefore, considering a meal with more SDS or RS will be healthier especially for diabetic people [4]. Postprandial level of blood glucose is generally estimated using a characteristic named glycemic index (GI) which is associated with the response of a consumed food to that of a reference one [5]. From the nutritional point of view, foods with lower GI value are considered as healthy ones which reduced the risk of many diseases such as diabetes, heart disease, some forms of cancers and so on [4, 6].

Starch modification which encompasses the alteration of physicochemical attributes of native starches can be exploited to improve their functionality [7-8]. Different chemical reactions are involved in chemical modification of starch like cross-linking, oxidation, etherification and esterification. Among these chemical methods, hydroxypropylation has been commonly used to improve the clarity, swelling power and retrogradation characteristics of native starches. One the other hand, cross-linking can strengthen the stability of the starch against specific conditions such as low pH, high temperature and shear [8]. It is well known that *in vitro* digestibility of native starches can be changed by physical and chemical modifications [9]. Over the two past decades, many studies have addressed the issue of influence of starch modification on its digestibility [3, 9-11]. In the case of hydroxypropylated starch, Wootton and Chaudhry (1989)[12]declared that substitution of bulky hydroxypropyl group causes a decrease *in vitro* digestibility of wheat

starch. For hydroxypropylated pea starch, Hoover et al. [13] found that by increasing the molar substitution (MS) up to 0.08, its digestibility decreased. Chung et al.[3] reported that among the modified corn starches, hydroxypropylated one showed the lowest digestibility values in gelatinized state. On the contrary, it has been observed that in granular state, by increasing the level of hydroxypropylation, a pronounced increase occurs in enzymatic digestibility due to the weakening of granular structure following chemical modification [14-16]. It is reported that in granular state, the cross-linking of starch with a mixture of sodium trimetaphosphate (STMP) and sodium tripolyphosphate (STPP) reduced the digestibility due to the enzymatic inhibitory effect [17]. Similar results were obtained for cross-linked corn starch, so that with increasing the amounts of cross-linking reagents (from 5 to 12%), the resistant starch increased up to 699.91% [9]. A drastic increase in digestibility of cross-linked corn starch was reported in the gelatinized state compared to the granular one [3].

Although many studies had been involved in investigation on digestibility of different starches (native or modified starches with different botanical resources), but in most of these studies, the digestibility has been obtained using the digestion procedure reported by Englyst et al.[1] or Koo et al.[9]. In this procedure to investigate the digestibility, gastrointestinal digestion conditions (the presence of saliva from the oral phase of digestion, acidic pH in the stomach and etc.) are not considered. Based on the literature review, no specific study was found to be associated with gastrointestinal digestion of modified wheat starches (study on both rheological and digestibility aspects). Therefore, the purpose of this investigation was to undertake a study of gastrointestinal digestion of native, cross-linked and hydroxypropylated wheat starches at two concentrations (8 and 12%) and volumes (7.5 and 15 ml), in three consecutive *in vitro* digestive stages, in which both rheological and digestibility aspects were considered.

2. Materials and methods

2.1. Materials

Native wheat starch (20±0.2% amylose) was purchased from Merck Company (Germany). Sodium trimetaphosphate (STMP), sodium tripolyphosphate (STPP), propylene oxide, 3,5-dinitrosalisylic acid, α-amylase (porcine pancreas, type VI-B, 10 U/mg solid), pepsin (porcine gastric mucosa, 400 U/mg protein), amyloglucosidase (*Aspergillus niger*, 70 U/mg), invertase (baker's yeast, 300 U/mg solid) and pancreatin (hog pancreas, 4× USP) were provided by Sigma Aldrich Company (St. Louis, MO). The other chemical materials used were of analytical grade.

2.2. Preparation of cross-linked and hydroxypropylated wheat starch samples

Cross-linked and hydroxypropylated wheat starch samples were prepared according to the method of Yousefi and Razavi[18]. In addition, degree of substitution (DS) for CLWS and HPWS were determined by the methods of Jackson[19] and Johnson[20], respectively. All of the samples were dispersed in two concentrations (8 and 12%, w/w) and volumes (7.5 and 15 ml) and completely gelatinized by cooking at 100 °C for 20 min in boiling water, and then cooled to room temperature (24±1 °C).

2.3. Determination of the starch fractions based on digestibility

In vitro starch digestibility of NWS, CLWS and HPWS was carried out by flowing method of Koo et al.[9] with minor modification. In brief, a pancreatic solution (1:12 w/w, pancreatin/distilled water) was prepared and centrifuged (1500×g, 10 min). Exactly 0.2 ml of amyloglucosidase was added to 10 ml of the separated supernatant, and the solute was reached to

volume of 12 with distilled water. Thirty mg of the starch samples and 0.75 ml of sodium acetate buffer (pH 5.2) was transferred into 2 ml micro tubes, and then shaken in a shaking incubator (37 °C, 10 min). Exactly 0.75 ml of the prepared amyloglucosidase solution was added to each micro tube and then incubated again (37 °C, 20 min). To prevent further enzymatic reaction, the micro tubes were taken from the incubator and placed in boiled water (~ 100 °C, 10 min). Finally, the glucose release concentration was measured using 3,5-dinitosalisylic acid (DNS) method. In addition, the rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) values were determined based on the amount of starch hydrolysis calculated from the following equation:

$$\%S_H = 0.9 \times G_R / S_i \qquad (1)$$

Where, $\%S_H$ is the percent starch hydrolysis, S_i the initial amount of starch, and G_R the amount of glucose release. A conversion factor of 0.9 was used due to the difference in starch monomer/molecular weight of glucose (162/180 = 0.9) [5].

2.4. Estimation of glycemic index (GI)

The following non-linear equation obtained by Goñi et al[21] was used to explain the starch hydrolysis kinetics.

$$C = C_\infty (1 - e^{-kt}) \qquad (2)$$

Where C refers to the amount of starch hydrolysis as percentage at time t, C_∞ is the equilibrium percentage of starch after 180 min enzymatic hydrolysis, K and t are the kinetic constant and hydrolysis time (min), respectively. Both C_∞ and K parameters were calculated for each starch sample on the basis of the obtained curve during 0 to 180 min hydrolysis.

The area under the obtained hydrolysis curve (AUC) was estimated based on the following equation:

$$AUC = C_{\infty}(t_f - t_0) - (C_{\infty} / k)[1 - \exp[-k(t_f - t_0)]] \qquad (3)$$

Where t_f is the final time (180 min) and t_0 is the initial time (0 min) of starch hydrolysis. Accordingly, the hydrolysis index (HI) was obtained by dividing AUC of each starch sample by the AUC of white bread as a reference. In the final step, the glycemic index (GI) was estimated according to the following equation as used by Chung et al.[3]:

$$GI = 39.71 + 0.549HI \qquad (4)$$

2.5. Saliva collection

The saliva employed in this experiment was completely fresh and collected from the same healthy donor according to the method of Yousefi and Razavi[22] as follows: to remove debris from the mouth of the donor, three times rinsing was done, and then to stimulate the secretion of saliva a sterilized nylon sheet (~5×5 cm^2) was used and the donor asked to chew it several times. Eventually, saliva was collected through spitting in a container and kept freshly in room temperature just before each experiment started. It should be noted that prior to the study, the donor gave his informed consent and this study was approved by the Ethics Committee of the Faculty of Agriculture, Ferdowsi University of Mashhad (FUM), Iran.

2.6. In vitro mouth and gastrointestinal models

To simulate the mouth model, the cooked starch samples at the mentioned concentrations and volumes were transferred in a 50 ml glass beaker and mixed with 2 ml of the collected fresh saliva for 5 s using a glass spatula with 1 cycle/s speed. The obtained starches-saliva mixtures

were immediately subjected to a two-stage model system consisting gastric and intestinal systems to show the gastrointestinal digestion of the used starches. Based on the US Pharmacopeia method with a simple modification, the simulated gastric and intestinal fluids (SGF and SIF) were prepared [23]. The starches-saliva mixtures (the starch samples were at two concentrations and volumes as declared in section 2.2) were added to 30 ml falcon tubes placed on a roller stirrer at 60 rpm. The roller mixer was placed on an incubator whose temperature was set at 37±1 °C accordant to the body temperature. The starch samples were digested for 40 min in 1.2 ml SGF containing pepsin (1.765:100, w/w (d. b.), pepsin/starch) at pH 1.2±0.05. The pH was consecutively checked (each 5 min) to maintain in the mentioned range using 0.5 M NaOH. After gastric digestion, the pH was changed to 6.8 by 1 M NaOH to hinder further digestion by pepsin. To simulate the intestinal digestion, 1.45 ml SIF containing pancreatin, invertase and amyloglucosidase with enzyme/starch (d. b.) ratio of 1.3:100, 1.1:1000 and 1:1000, w/w, respectively was added to the falcon tubes. The simulated intestinal digestion was done for 120 min while the mixtures were stirred at 60 rpm at 37±1 °C and the pH was adjusted at 6.8±0.05 over the digestion period. Aliquots (0.5 ml) were removed at 0, 5, 10, 15, 20, 30 and 40 min of digestion from the simulated gastric condition (SGC) and at 1, 2, 5, 10, 15, 20, 30, 45, 60, 80, 90, 100 and 120 min of digestion from the simulated intestinal condition (SIC) and eventually prepared for reducing sugar measurement.

2.7. Determination of reducing sugars

To hamper further enzymatic hydrolysis, the withdrawn aliquots at the mentioned times of digestion were immediately mixed with 2.5 ml of absolute ethanol. These mixtures were equilibrated to room temperature (24±1 °C) for 30 min. Then, to convert all the sugars produced by enzymatic hydrolysis to glucose, 0.1 ml of the mixtures were taken and then incubated at

187 37±1 °C for 10 min with the mixture of invertase and amyloglucosidase enzymes in acetate
188 buffer with pH 5.2 (1 mg amyloglucosidase and 0.4 mg invertase per 10 ml buffer). Finally, the
189 extent of glucose (released or obtained by enzymatic conversion) was measured using 3,5-
190 dinitosalicylic acid method and expressed as mg glucose/100 ml of digested mixtures [24].
191
192 **2.8. Rheological measurements**
193 The rheological measurements were performed using a rotational viscometer (Bohlin Model
194 Visco 88, Bohlin Instruments, UK). Appropriate bob and cup measuring (C30) was selected
195 based on the viscosity of the obtained dispersion. Steady shear flow behavior of the native and
196 hydroxypropylated starch samples at concentration of 8% and volume of 7.5 ml, exactly after
197 digestion in the simulated gastric and intestinal conditions was obtained at 25 °C in a strain-
198 controlled mode. Shear stress *vs.* shear rate data within the range of 20 to 220 s^{-1} was collected.
199 Then to describe the flow properties of the samples, the Power law (or Ostwald-Waele's) model
200 was used (Eq. 5) [25]:
201 $$\tau = k\dot{\gamma}^n \quad (5)$$
202 where *k* and *n* are the consistency coefficient ($Pa.s^n$) and the flow behavior index, respectively.
203
204 **2.9. Statistical Analysis**
205 Statistical analyses were conducted using SPSS 17 (SPSS Inc., Chicago, IL, USA). The obtained
206 data were analyzed by one-way analysis of variance (ANOVA) at 95% confidence level. The
207 independent t-test was used for all combinations of two data sets at 95% confidence level.
208 Determination coefficient (R^2) was applied as a criterion to evaluate the performance of models
209 used. Data were obtained in triplicate and presented as the mean ± standard deviation.

3. Results and discussion

3.1. Enzymatic hydrolysis and starch fractions based on digestibility

The phosphorous and hydroxypropyl contents in CLWS and HPWS were found to be 0.096 and 2.106%, respectively. Fig. 1 shows the digestion behavior of the gelatinized NWS, CLWS and HPWS. It can be seen that 92.02% of NWS, 91.31 % of CLWS and 86.09% of HPWS hydrolysis occurred at the early 20 min of digestion, and after that the extent of hydrolysis reached plateaus. As a result, no significant difference was observed between the amounts of hydrolysis of NWS and CLWS ($p<0.05$). In contrast, introducing hydroxypropyl groups has significantly affected the enzymatic hydrolysis of NWS ($p<0.05$), so that after 180 min, the extent of hydrolysis for HPWS was 7.34% lower than that of NWS. The hydroxypropyl groups on starch chains act as a physical obstacle which hamper the enzymatic hydrolysis and also make the adjacent bonds resistance to degradation[10]. Östergård et al.[26] found lesser susceptibility of gelatinized hydroxypropylated potato starch to enzymatic attack even than acetylated potato starch because of higher bulky configuration. The similar results were reported for pea starch [13], corn starch [3] and waxy and non-waxy rice starches [27]. Table 1 represents the RDS, SDS and RS contents of the gelatinized NWS, CLWS and HPWS obtained based on the hydrolysis pattern of the starch samples. It was observed that the RDS content of NWS (92.02%) and CLWS (91.31%) were significantly ($p<0.05$) higher than that of HPWS (86.09%), while these results were conversely for the RS content (6.59, 7.57 and 13.15% for NWS, CLWS and HPWS, respectively). The RS content of native wheat starch was found to be lesser than smooth pea starch [28], normal corn starch [3],

banana starches [29] and cassava starch [30], but higher than sweet corn and potato starches [30]. HPWS gel samples showed significantly lower SDS content (0.76%) than the NWS (1.39%) and CLWS (1.12%) ones ($p<0.05$). These values of SDS content demonstrate a little enzymatic hydrolysis for the time interval between 20 and 180 min of digestion. It is obvious that this issue is due to the rapid enzymatic hydrolysis of the starch samples in the first 20 min of digestion time. As a result, it could be said that by cross-linking (0.096%) and hydroxypropylation (2.106%) of NWS, about 0.98 and 6.56% of RDS values may be partially transformed to RS ones, respectively. Hwang et al.[27] reported that by using 10% propylene oxide for production of hydroxypropylated starch from normal rice starch with 3.06 RS content, the RS level increased to 20.03%. The RS content of hydroxypopylated normal corn [3] and waxy rice [27] starches were reported to be19.5% and 4.58%, respectively. It should be noted that the declared results were only for the gelatinized starch form while the observed trends were reversed in granular phase [12, 14, 16, 26]. Even higher RDS value for gelatinized cross-linked corn starch (93.0%) than native one (92.7%) was reported by Chung et al.[3].

3.2. Kinetics of starch digestion and estimated glycemic index

Table 2 shows the hydrolysis index (HI), estimated glycemic index (GI) and calculated parameters from equation 2, describing the kinetics of NWS, CLWS and HPWS digestion. The equilibrium concentration (C_∞) which is related to the constant amount of hydrolysis calculated from the plateau part of hydrolysis curve was 92.50, 91.06 and 85.75% for NWS, CLWS and HPWS, respectively. This parameter indicated more susceptibility of NWS and CLWS compared to HPWS to enzymatic hydrolysis by α-amylase in the middle and end stages of digestion. The kinetic constant (k) values of NWS, CLWS and HPWS were significantly different ($p<0.05$).

These differences might be due to the rate of enzyme attack by the different degrees of swelling [3]. Many studies have reported more swelling degree of HPWS than NWS and CLWS [8]. Based on equation 3, the *AUC* extent for NWS, CLWS and HPWS was estimated and then converted to the HI and GI. As shown in Table 2, the HI of NWS (97.31) was higher than that of CLWS (95.61) and HPWS (89.86) and accordingly, higher GI was estimated for NWS (93.13) in comparison with CLWS (92.20) and HPWS (89.04). These results were in agreement with the results reported by Björck et al.[10] in which they found that the digestibility of potato starch was significantly reduced by hydroxypropylation. A GI value lower than 100 (correspond to white bread as a reference) indicated lesser ability of the starches tested to raise the blood glucose level than the white bread.

3.3. In vitro gastrointestinal digestion

Gastrointestinal hydrolysis of NWS, CLWS and HPWS at different concentrations and volumes are shown in Fig. 2. No specific hydrolysis was observed during the SGC (0-40 min) due to the lack of starch-hydrolysing enzymes and acidic deactivation of salivary amylase added in previous digestion stage. Although more amounts of glucose release was attained for NWS than CLWS and HPWS at the SGC for each concentration and volume tested, but the differences were not significant ($p>0.05$). It is obvious that some determined hydrolysis at the gastric stage has been due to the acid hydrolysis at low pH (1.2). Previous studies have been declared that no significant digestion of carbohydrates take place in gastric condition [24, 31]. It was found the extents of glucose release at SGC were significantly affected by the concentrations used ($p<0.05$), whereas no significant differences were observed affected by the volumes tested ($p>0.05$).

After adding the simulated intestinal fluid (SIF) to the reaction mixtures, a drastic digestion was occurred by the pancreatic amylases (Fig. 2). As a result, 82-87, 76-81 and 77-84% of the final glucose release were obtained within first 15 min after digestion at SIC for the NWS, CLWS and HPWS samples, respectively. The highest maximum glucose release level was for NWS (9.27 mg/100 ml, at concentration 12% and volume 15 ml), while the lowest maximum of that obtained for HPWS (5.17 mg/100 ml, at concentration 8% and volume 7.5 ml). There were significant differences in glucose release extents between the all samples experimented ($p<0.05$), and the NWS samples had higher glucose release over the digestion time at SIC. According to the results, the amounts of glucose release for CLWS and HPWS were approximately 6-11 and 16-19% lower than those for NWS at the end of digestion at SIC. These results were in agreement with the results obtained *in vitro* digestion based on Koo et al.[9] method (Table 1) in which higher RS value was calculated for HPWS (13.15%) than CLWS (7.57%) and NWS (6.59%). Increasing in concentration and volume resulted in an increase in glucose release, but the impact of concentration was more pronounced ($p<0.05$). Accordingly, with increasing concentration 34.78-48.94, 39.39-45.90 and 38.23-43.40%, and with increasing volume 30.77-33.46, 23.23-28.98 and 21.84-26.40% increase in amounts of glucose release at the end of digestion at SIC were obtained for the NWS, CLWS and HPWS samples, respectively.

To declare the behavior of glucose release from each starch sample at SGC and SIC (glucose release *vs.* digestion time), different models were used. In brief, it was observed that the linear equations were fitted well to the experimental data at SGC, whereas the amounts of glucose release at SIC were appropriately modeled by the two-term exponential model (Table 3). The values of R^2 calculated using regression analysis of the linear ($y=a.x+b$) and two-term exponential ($y=a.\exp(b.x)+c.\exp(d.x)$) models were within the range of 0.858-0.987 and 0.938-

0.991, respectively. The slope of linear changes in glucose release at SGC were 0.007-0.014, 0.001-0.005 and 0.007-0.011 for the NWS, CLWS and HPWS samples, respectively, which increased with increasing volume and concentration. On the other hand, at SIC, the changes in the "a" exponent of the two-term exponential model showed the amounts of glucose release in plateau state, which were 4.99-9.48, 4.46-9.73 and 4.19-7.71 (mg/100 ml) for the NWS, CLWS and HPWS, respectively. There were no specific investigations on modeling of gastrointestinal glucose release from the gelatinized native or modified starches, whether *in vitro* or *in vivo* experiments, but several studies were found to be involved in modeling of intestinal glucose absorption [32-33], disintegration kinetics of solid foods during gastric digestion [34] and elution profile of sodium caseinate [35]. It is important to note that the observed digestion behaviors and the obtained glucose release extents were in a simple digestive system included only each starch sample separately, so the calculated amounts of glucose release both at SGC and SIG cannot be interpreted as the *in vivo* results for starchy foods, because they are much more complex. Based on the digestion results of the starch samples (Table 1 and Fig.1), it is obvious that the HPWS contains more RS. The foodstuffs with high contents of RS have some putative health benefits such as decreasing the postprandial blood glucose content [36], producing more nutrition components by microbial fermentation in the large bowl [37] and ability to control the initiation of colonic cancer [4].

3.4. Rheological properties

3.4.1. Steady shear flow behavior after digestion at SGC

Fig. 3 shows the typical flow curve of NWS, CLWS and HPWS at the end of digestion (after 40 min) at SGC in presence and absence of acidic pH. As it can be seen, the steady shear viscosity

of NWS was higher than that of CLWS and HPWS in both conditions. For instance, the apparent viscosity of NWS were 595.5, 938.5, 741.9 and 853.3% higher than those for HPWS in acidic pH at shear rates of 25, 50, 100 and 200 s^{-1}, respectively. At these noted shear rates, approximately 43.00, 44.54, 59.65 and 68.00% decrease in apparent viscosity was observed for NWS, 40.91, 42.59, 54.83 and 58.82% for CLWS, and also 67.32, 78.33, 77.86 and 85.00% for HPWS in comparison with the undigested (before using at digestion process) gel samples at 37 °C (data not shown). These results indicated higher shear stability and sensitivity of CLWS and HPWS than NWS at SGC, respectively. An apparent shear-thinning (pseudoplastic) behavior was observed for both starch samples. It was found that the acidic pH (1.2) had no significant impact on apparent viscosity as compared with the control samples ($p>0.05$). As declared before, this issue is associated with the lack of starch-hydrolyzing enzymes at gastric fluid. The rheological parameters of the samples at SGC attained by fitting the collected data (shear stress *vs.* shear rate) to the Ostwald-Waele's model at different conditions are given in Table 4. It was found that the obtained mixtures after digestion of the starch samples at SGC had lower consistency *(k)* and flow behavior index *(n)* values than undigested samples. In brief, the decreased values in *k* and *n* parameters were 18.12 and 41.38% for NWS, 30.78 and 57.89% for CLWS, and also 33.50 and 60.71% for HPWS, respectively. So, more rheological changes (lower consistency and higher flowability) were attained for the HPWS samples than others. The main reason for the observed decrease in *n* values (more pseudoplasticity) at SGC may be due to the pseudoplastic behavior of saliva added in the previous stage [38]. As it is seen, no specific changes were observed in the Power law model parameters (*k* and *n*) affected by the use of acidic pH. Therefore, it can be concluded that the dilution effect of saliva and SGF added were the main possible reasons for the observed decrease in apparent viscosity of all the samples

348 experimented. Majzoobi and Beparva[39] reported that the lactic and acetic acids had no definite
349 influence on intrinsic viscosity of native and cross-linked wheat starch gels.
350
351 **3.4.2. Steady shear flow behavior after digestion at SIC**
352 All of the obtained samples after passing through the SIC (in the presence or absence of the
353 enzymes) exhibited shear-thinning (pseudoplastic) behavior (Fig. 4). Rao[40] declared that this
354 flow behavior is the typical one for many polymer solutions. More decrease in apparent viscosity
355 of the NWS, CLWS and HPWS samples was observed in the presence of starch-hydrolysing
356 enzymes (amyloglucosidase, invertase and pancreatine), indicating the pronounced impact of the
357 enzymes ($p<0.05$). Besides, a significant decrease in apparent viscosity of the NWS, CLWS and
358 HPWS samples was seen at SIC as compared with the results obtained at SGC ($p<0.05$). As a
359 result, after simulated intestinal digestion approximately 85.00, 81.82, 84.38 and 88.75%
360 decrease in apparent viscosity was obtained for the NWS; 57.95, 51.85, 61.29 and 64.70% for
361 the CLWS; and finally 92.44, 92.50, 92.86 and 93.40% for the HPWS samples in comparison
362 with the undigested starch samples at the selected shear rates (25, 50, 100 and 200 s^{-1},
363 respectively). Increase in viscosity cause to decrease in amount of enzymatic products not only
364 through hindering the enzyme-substrate contact, but also by affecting the enzymatic kinetics [23,
365 41]. Although many studies have been declared that lower viscosity resulted in more digestibility,
366 but in case of wheat starch it was found that substitution of hydroxypropyl groups (2.106%) had
367 more enzymatic inhibitory effect than shear viscosity. The *n* and *k* values obtained for the
368 mixtures after 120 min digestion at SIC (in the presence and absence of the enzymes) based on
369 the Ostwald-Waele's model are shown in Table 4. Comparing these parameters obtained at SIC
370 (in the presence of the enzymes) with those at SGC showed that a drastic decrease in the *k* values

occurred while the *n* values increased. Therefore, 90.27, 73.02 and 89.77% decrement in the *k* values and 223.53, 155.56 and 363.64% increment in the *n* value was observed for the NWS, CLWS and HPWS samples, respectively. As it can be seen, the most and least changes in the rheological parameters were related to the HPWS and CLWS, respectively, indicating the impact of chemical modifications on rheological characteristics at SIC. An issue that should be underlined is that the lesser changes obtained for the *k* (50.78-64.02%) and *n* (70.59-154.55%) values in the absence of the enzymes (the control samples) were due to the dilute effect of the SIF and applied shear stress by stirring at SIC (60 rpm at 37 °C). Similar results was reported by Dartois et al.[23] for cooked waxy corn starch except that higher *n* values reported by them at SIC. This could be for two reasons; one due to using different units of the enzymes in the SIF and the other, disregarding the inhibitory role of mucin in the saliva which behaves like a barrier against further hydrolyzing [42-43]. The role of mucin was not considered in their study, because their simulation was begun from the gastric condition.

4. Conclusions

The estimated glycemic index and gastrointestinal digestibility of starch could be affected by the chemical modifications. Hydroxypropylation reduced starch digestibility and raised the extent of resistant starch (RS) by decreasing the rapidly digestible starch (RDS) content, whereas cross-linking had no specific influence on these fractions. Simulated gastric conditions (SGC) had not specific impact on the amount of glucose release, whereas a drastic increase of that was obtained at simulated intestinal conditions (SIC) influenced by the starch-hydrolyzing enzymes. It was found that these intestinal enzymes had more pronounced effect on the rheological characteristics

(consistency coefficient and flow behavior index) of NWS, CLWS and HPWS as compared with the acidic pH at SGC. It should be noted that in such simulated experiments, a simple condition was assumed which is not completely coincide to the physiological condition, so because of more complex condition *in vivo* these results could not be used as real data. For example, *in vivo* system the presence of viscous mucins may play a significant role on hydrolysis kinetics.

References

1. H. N. Englyst, S. M. Kingman and J. H. Cummings, *European Journal of Clinical Nutrition*, 1992, **46**, 33-50.
2. U. Lehmann and F. Robin, *Trends in Food Science & Technology*, 2007, **18**, 346-355.
3. H.-J. Chung, D.-H. Shin and S.-T. Lim, *Food Research International*, 2008, **41**, 579-585.
4. A. Perera, V. Meda and R. T. Tyler, *Food Research International*, 2010, **43**, 1959-1974.
5. M. Frei, P. Siddhuraju and K. Becker, *Food Chemistry*, 2003, **83**, 395-402.
6. R. K. Le Leu, Y. Hu, I. L. Brown and G. P. Young, *Nutr Metab (Lond)*, 2009, **6**, 11.
7. L. Jayakody and R. Hoover, *Food Research International*, 2002, **35**, 665-680.
8. J. Singh, L. Kaur and O. McCarthy, *Food Hydrocolloids*, 2007, **21**, 1-22.
9. S. H. Koo, K. Y. Lee and H. G. Lee, *Food Hydrocolloids*, 2010, **24**, 619-625.
10. I. Björck, A. Gunnarsson and K. Østergård, *Starch - Stärke*, 1989, **41**, 128-134.
11. S. I. Shin, H. J. Kim, H. J. Ha, S. H. Lee and T. W. Moon, *Starch/Stärke*, 2005, **57**, 421-430.
12. M. Wootton and M. Chaudhry, *Starch/Stärke*, 1981, **33**, 135-137.
13. R. Hoover, D. Hannouz and F. Sosulski, *Starch/Stärke*, 1988, **40**, 383-387.
14. H. Liu, L. Ramsden and H. Corke, *Carbohydrate Polymers*, 1999, **40**, 175-182.
15. O. S. Lawal, *Food Hydrocolloids*, 2009, **23**, 415-425.
16. O. S. Lawal, *LWT-Food Science and Technology*, 2011, **44**, 771-778.
17. K. Woo and P. Seib, *Cereal Chemistry*, 2002, **79**, 819-825.
18. A. R. Yousefi and S. M. A. Razavi, *Starch/Stärke*, 2015, [accepted paper].

19. M. L. Jackson, ed. E. M. L. Jackson, Prentice Hall of India, Pvt. Ltd, New Delhi, 1967, ch. 151-154.
20. D. P. Johnson, *Analytical Chemistry*, 1969, **41**, 859-860.
21. I. Goñi, A. Garcia-Alonso and F. Saura-Calixto, *Nutrition Research*, 1997, **17**, 427-437.
22. A. R. Yousefi and S. M. A. Razavi, *Journal of Food Process Engineering*, 2015, DOI: 10.1111/jfpe.12196.
23. A. Dartois, J. Singh, L. Kaur and H. Singh, *Food Biophysics*, 2010, **5**, 149-160.
24. A. Bordoloi, J. Singh and L. Kaur, *Food Chemistry*, 2012, **133**, 1206-1213.
25. M. A. Rao, *Rheology of fluid and semisolid foods; principles and applications*, USA: Aspen Publishers, Inc., 1999.
26. K. Östergård, I. Björck and A. Gunnarsson, *Starch - Stärke*, 1988, **40**, 58-66.
27. D.-K. Hwang, S.-W. Kim, J.-H. Kim, J.-H. Ryu, S.-H. Yoo, C.-S. Park, B.-Y. Kim and M.-Y. Baik, *Starch - Stärke*, 2009, **61**, 20-27.
28. W. S. Ratnayake, R. Hoover, F. Shahidi, C. Perera and J. Jane, *Food Chemistry*, 2001, **74**, 189-202.
29. N. Vatanasuchart, B. Niyomwit and K. Wongkrajang, *Maejo International Journal of Science and Technology*, 2012, **6**, 259-271.
30. A. Moongngarm, *American Journal of Agricultural and Biological Sciences*, 2013, **8**, 107-113.
31. J. W. Woolnough, J. A. Monro, C. S. Brennan and A. R. Bird, *International Journal of Food Science & Technology*, 2008, **43**, 2245-2256.
32. S. Salinari, A. Bertuzzi and G. Mingrone, *American Journal of Physiology-Endocrinology and Metabolism*, 2011, **300**, E955-E965.
33. A. A. Gruzdkov, L. V. Gromova, N. M. Grefner and Y. Y. Komissarchik, *Journal of Biophysical Chemistry*, 2012, **3**.
34. F. Kong and R. Singh, *Journal of food science*, 2008, **73**, E202-E210.
35. M. Nakauma, S. Nakao, S. Ishihara and T. Funami, *Food Hydrocolloids*, 2014, **36**, 294-300.
36. C. W. Kendall, A. Esfahani, A. J. Hoffman, A. Evans, L. M. Sanders, A. R. Josse, E. Vidgen and S. M. Potter, *Journal of the American College of Nutrition*, 2008, **27**, 711-718.

37. J. M. Wong, R. de Souza, C. W. Kendall, A. Emam and D. J. Jenkins, *Journal of clinical gastroenterology*, 2006, **40**, 235-243.
38. J. R. Stokes and G. A. Davies, *Biorheology*, 2007, **44**, 141-160.
39. M. Majzoobi and P. Beparva, *Food Chemistry*, 2014, **147**, 312-317.
40. M. A. Rao, *Rheology of Fluid and Semisolid Foods: Principles and Applications*, Springer, New York, 2007.
41. C. L. Dikeman, M. R. Murphy and G. C. Fahey, *The Journal of nutrition*, 2006, **136**, 913-919.
42. S. J. Haward, J. A. Odell, M. Berry and T. Hall, *Rheologica acta*, 2011, 50, 869-879.
43. H. Choi, J. R. Mitchell, S. R. Gaddipati, S. E. Hill and B. Wolf, Food Hydrocolloids, 2014, 40, 71-75.

Table 1. Amounts of rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistance starch (RS) of native (NWS), cross-linked (CLWS) and hydroxypropylated wheat starches (HPWS)*

Starch	RDS%	SDS%	RS%
NWS	92.02±1.70a	1.39±0.17a	6.59±1.67b
CLWS	91.31±0.62a	1.12±0.09b	7.57±1.58b
HPWS	86.09±0.89b	0.76±0.06c	13.15±1.97a

*Values followed by a different letter in each column are significantly different ($p < 0.05$).

Table 2. Hydrolysis index (HI), estimated glycemic index (GI), equilibrium concentration (C_∞) and kinetic constant (k) for native (NWS), cross-linked (CLWS) and hydroxypropylated wheat starches (HPWS)*

Starch	HI	GI	C_∞	k	R^2
NWS	97.31±0.24a	93.13±0.19a	92.50±0.67a	0.239±0.019b	0.976
CLWS	95.61±0.21a	92.20±0.16a	91.06±0.72a	0.221±0.017c	0.948
HPWS	89.86±0.16b	89.04±0.13b	85.75±0.64b	0.250±0.027a	0.925

*Values followed by a different letter in each column are significantly different ($p < 0.05$).

524 Table 3. The equations of the best fitted models to the experimental results of glucose release at SGC and SIC for native (NWS), cross-linked
525 (CLWS) and hydroxypropylated wheat starches (HPWS)

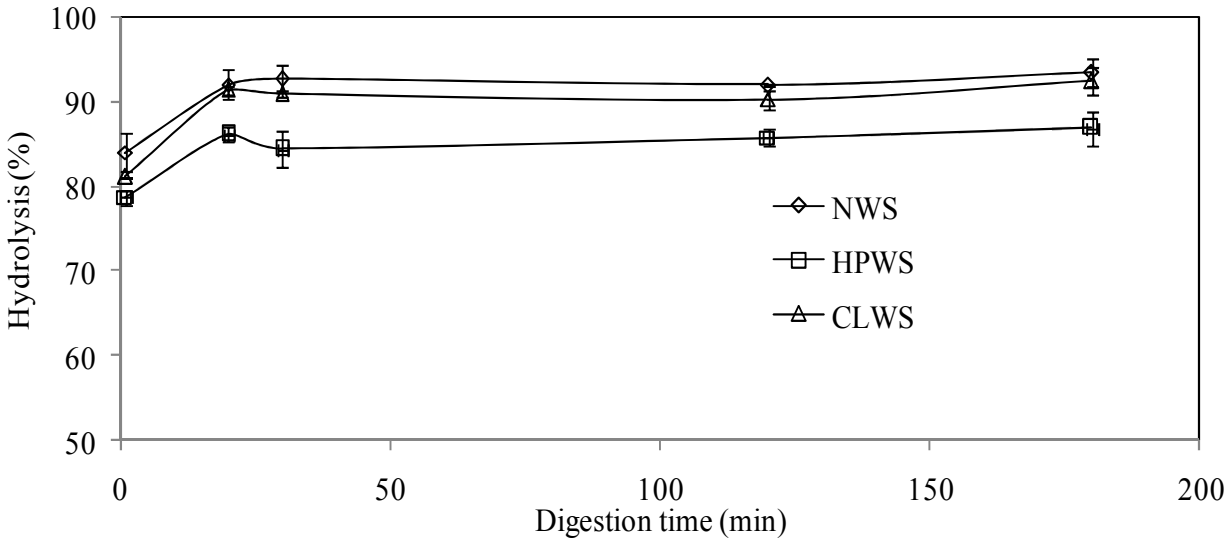
Simulated condition	Sample	7.5 ml		15 ml	
		Equation	R^2	Equation	R^2
SGC	NWS-8%	0.007x+0.084	0.981	0.009x+0.076	0.972
	NWS-12%	0.01x+0.131	0.956	0.014x+0.198	0.914
	CLWS-8%	0.001x+0.121	0.932	0.003x+0.110	0.858
	CLWS-12%	0.005x+0.139	0.940	0.005x+0.159	0.872
	HPWS-8%	0.007x+0.054	0.986	0.007x+0.050	0.987
	HPWS-12%	0.009x+0.082	0.975	0.011x+0.136	0.931
SIC	NWS-8%	4.99exp(0.01x)-0.04exp(-3.87x)	0.991	6.52exp(0.02x)-0.04exp(-4.17x)	0.978
	NWS-12%	7.22exp(0.03x)-0.05exp(-4.20x)	0.989	9.48exp(-0.03x)-0.33exp(-2.34x)	0.983
	CLWS-8%	4.46exp(0.001x)-2.74exp(-0.08x)	0.989	5.81exp(0.003x)-3.61exp(0.09x)	0.984
	CLWS-12%	6.8exp(-0.0001x)-4.8exp(-0.1x)	0.983	9.73exp(-0.001x)-4.4exp(-0.04x)	0.938
	HPWS-8%	4.19exp(0.06x)-0.01exp(-5.28x)	0.981	5.39exp(0.01x)-0.07exp(-3.78x)	0.987
	HPWS-12%	6.29exp(-0.01x)-0.19exp(-3.03x)	0.967	7.71exp(-0.01x)-0.04exp(-3.89x)	0.951

526

Table 4. Effect of acidic pH and hydrolyzing enzymes on Ostwald-Waele's model parameters for digestion of native (NWS), cross-linked (CLWS) and hydroxypropylated wheat starches (HPWS) at SGC and SIC

Simulated condition	Sample	*k*	*n*	R^2	RMSE
Undigested	NWS	14.07±0.86	0.29±0.01	0.962	0.090
	CLWS	13.35±0.54	0.19±0.02	0.999	0.009
	HPWS	3.97±0.61	0.28±0.01	0.974	0.020
SGC	NWS-control	12.28±0.11	0.18±0.03	0.978	0.051
	HPWS-control	2.69±0.24	0.11±0.01	0.979	0.005
	CLWS-control	10.74±0.08	0.10±0.01	0.981	0.026
	NWS+ acidic pH	11.52±0.43	0.17±0.02	0.990	0.031
	CLWS+ acidic pH	9.24±0.12	0.18±0.02	0.989	0.017
	HPWS+ acidic pH	2.64±0.19	0.11±0.01	0.966	0.005
SIC	NWS-control	5.67±0.39	0.29±0.02	0.963	0.035
	HPWS-control	0.95±0.08	0.28±0.01	0.959	0.005
	CLWS-control	3.67±0.22	0.31±0.04	0.988	0.013
	NWS+ enzymes	1.12±0.14	0.55±0.03	0.951	0.018
	CLWS+ enzymes	0.99±0.14	0.46±0.05	0.971	0.007
	HPWS+ enzymes	0.27±0.06	0.51±0.04	0.958	0.007

537



538
539

540 **Figure 1.**

541

542

543

544

545

546

547

548

549

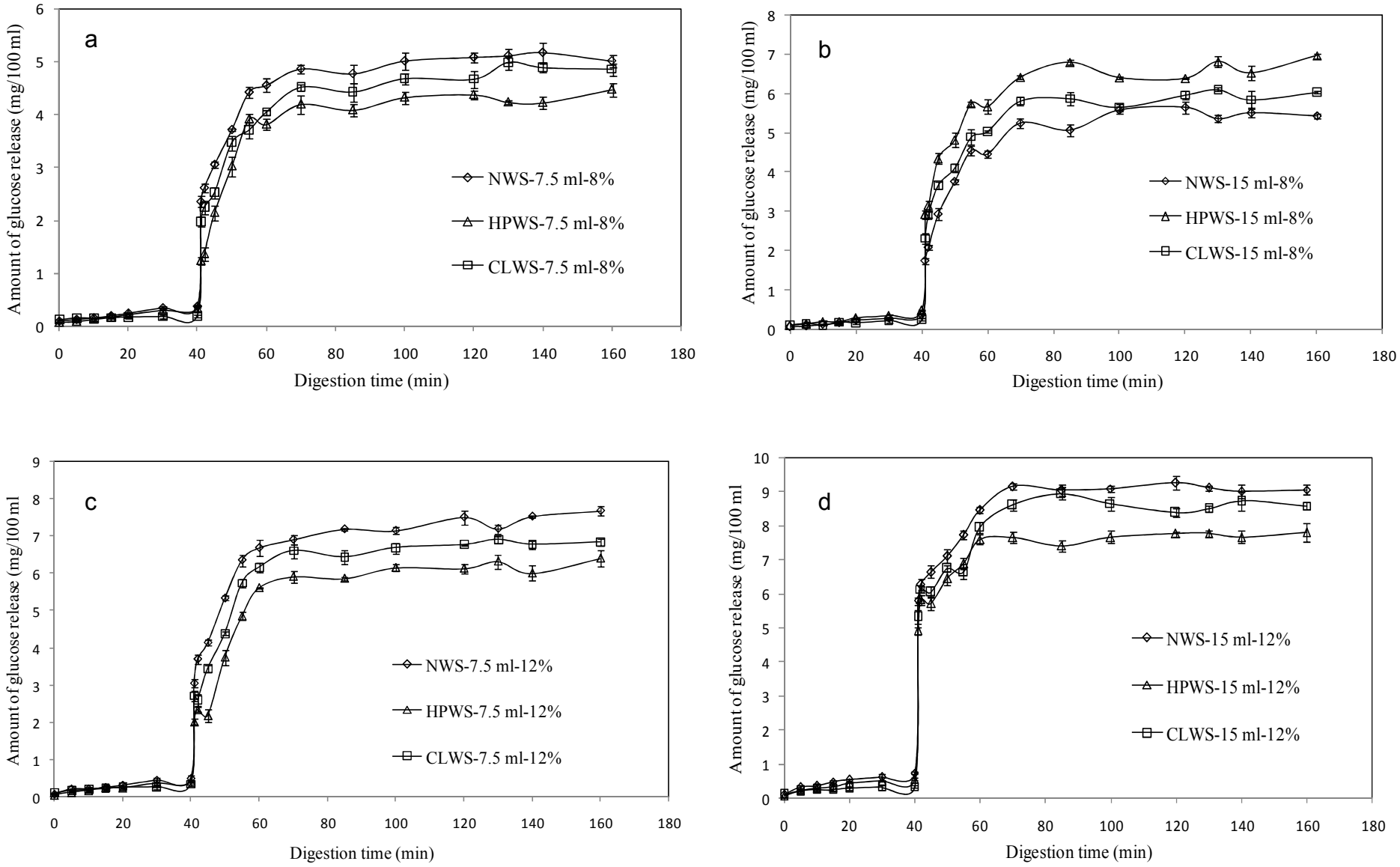
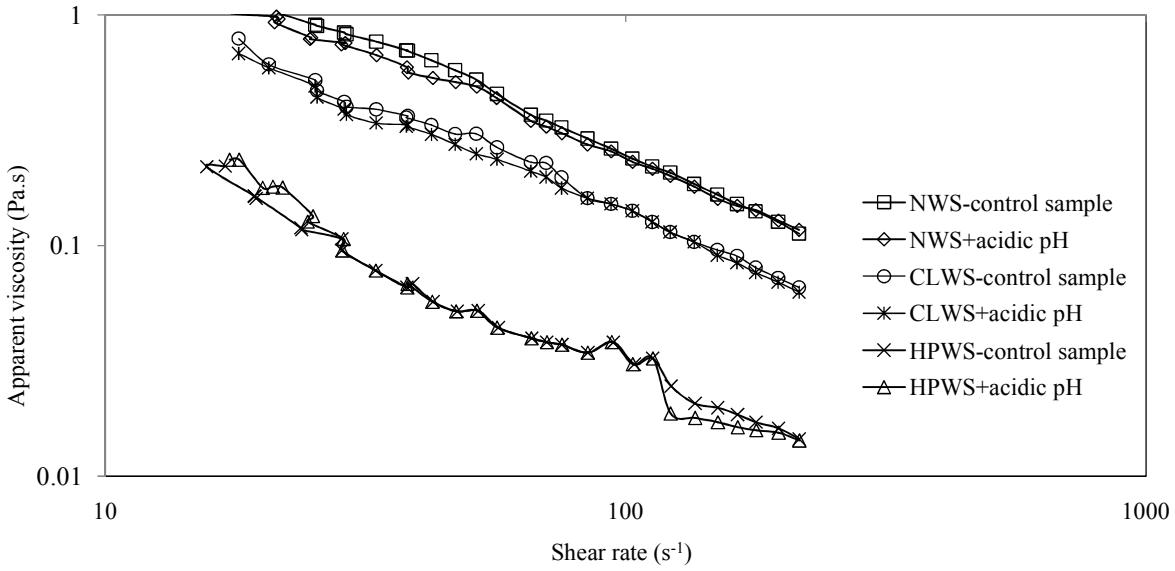


Figure 2.

553



554
555

556 **Figure 3.**

557

558

559

560

561

562

563

564

565

566

567

568

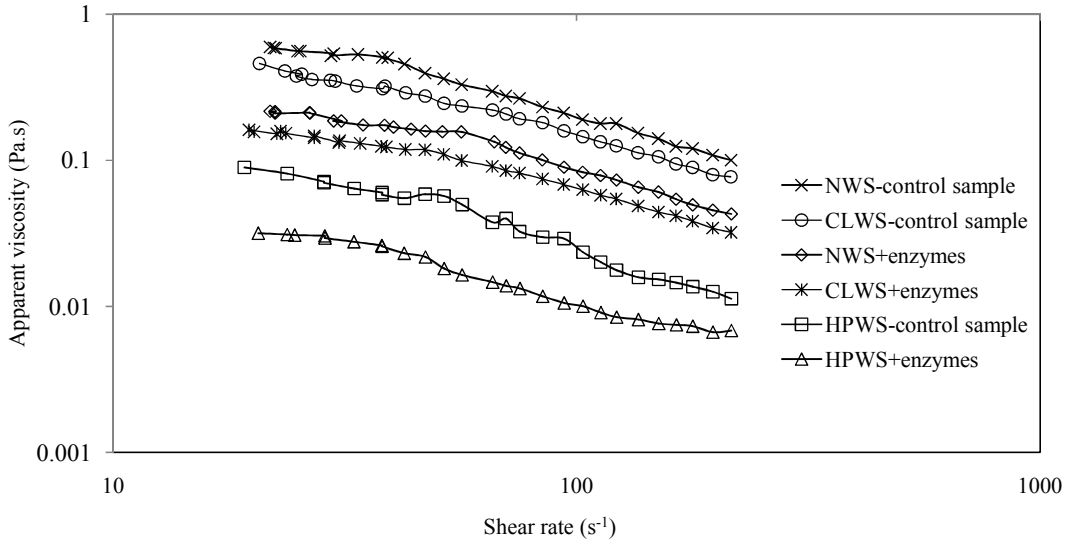


Figure 4.

585 **Figure captions**

586

587 **Figure 1.** Starch hydrolysis pattern of native (NWS), cross-linked (CLWS) and
588 hydroxypropylated wheat starches (HPWS) using pancreatic α-amylase for 3h at 37 °C (The first
589 data are corresponding to the hydrolysis at time of 1 min).

590 **Figure 2.** Amount of glucose release from native (NWS), cross-linked (CLWS) and
591 hydroxypropylated wheat starches (HPWS) at different volumes and concentrations (a) 7.5 ml
592 and 8%, (b) 15 ml and 8%, (c) 7.5 ml and 12%, (d) 15 ml and 12%. (Digestion times between 0-
593 40 and 40-160 are related to digestion at SGC and SIC, respectively).

594 **Figure 3.** Effect of acidic pH on viscous flow curves of native (NWS), cross-linked (CLWS) and
595 hydroxypropylated wheat starches (HPWS) after digestion at SGC.

596 **Figure 4.** Effect of hydrolyzing enzymes on viscous flow curves of native (NWS), cross-linked
597 (CLWS) and hydroxypropylated wheat starches (HPWS) after digestion at SIC.

598

599