In vitro digestion of pectin- and mango-enriched diets using a dynamic rat stomach-duodenum model

Peng Wu, Rewati R. Bhattarai, Sushil Dhital, Renpan Deng, Xiao Dong Chen, Michael J. Gidley

PII: S0260-8774(17)30011-0
DOI: 10.1016/j.jfoodeng.2017.01.011
Reference: JFOE 8758

To appear in: Journal of Food Engineering

Received Date: 16 March 2016
Revised Date: 9 January 2017
Accepted Date: 12 January 2017


This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
In vitro digestion of pectin- and mango-enriched diets using a dynamic rat stomach-duodenum model

Peng Wu a, b, Rewati R Bhattarai b, Sushil Dhital b, Renpan Deng a, Xiao Dong Chen a, c, *, Michael J. Gidley b, *

a Department of Chemical Engineering and Biochemical Engineering, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, China
b ARC Centre of Excellence in Plant Cell Walls, Centre for Nutrition and Food Sciences, Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St Lucia, 4072, QLD, Australia
c School of Chemical and Environmental Engineering, College of Chemistry, Chemical Engineering and Materials Science, Soochow University, Suzhou 215123, China

* Corresponding authors

Phone: +61 7 3365 2145; Fax: +61 7 3365 1177. Email address: m.gidley@uq.edu.au (M. J. Gidley).
Phone: +86 592 2189073; Fax: +86 592 2188855. Email address: xdc@xmu.edu.cn (X.D. Chen).
Abstract

In this study we investigated the \textit{in vitro} digestive behaviors of starch-based diets enriched with soluble fiber (pectin) and fruit powder (mango) varying with dry matter contents using a dynamic rat stomach and duodenum model. The changes in pH with respect to digestion time, gastric emptying, starch and protein hydrolysis as well as rheological and microstructural properties of the gastric and duodenal digesta were investigated. The effects of porcine gastric mucin on digestion were studied as well. The results showed that gastric digestion and emptying rate were decreased with increasing dry matter content of the diets. Both the dynamic and steady shear viscosity of the duodenal digesta was lower than the gastric digesta due to hydrolysis of starch and protein as well as dilution by added digestive juice. With the same dry matter content (20.35%), the rates of gastric emptying as well as starch and protein hydrolysis were higher in the control and mango diets compared to the pectin diet, illustrating the complex relationships among rheology, soluble dietary fiber and digestion. The addition of gastric mucin could delay the rates of gastric digestion and emptying by increasing digesta viscosity. The study emulates the complexity of heterogeneous diets during simulated gastric and duodenal digestion and suggests that the viscosity generated by either addition of mucin on the diet or naturally exerted by e.g. pectin could be the rate limiting factor for both gastric emptying and hydrolysis of starch and proteins.

Key words: DIVRSD model; Rolling extrusion; Digestion; Gastric emptying rate; Pectin; Porcine gastric mucin
1. Introduction

Numerous in vitro gastrointestinal (GI) models have been developed in the past few decades, which are widely used to test the structural and physical/chemical changes that occur in different foods under simulated GI conditions. Compared with in vivo studies conducted on animals or human volunteers, in vitro models can provide advantages in saving time and cost, better repeatability and the possibility of collecting samples at any position within the (simulated) GI tract at any time during digestion (Chen, 2006; Hur et al., 2010; Wu et al., 2014). Another advantage of in vitro techniques is that they are not impeded by ethical constraints that often limit human or animal experimentation (Blanquet et al., 2004). There are two types of GI tract models available in the food digestion area, namely static and dynamic models. The static models do not mimic the physical and physiological processes that occurs in vivo, such as pH change and peristaltic movements. Moreover, the physical structure and physicochemical characteristics of food and the removal of digesta are rarely considered when simulating food digestion. Many in vitro static models mimic food digestion processes by simply mixing food and gastric fluid under simulated gastric conditions for 30 to 120 min followed by (sampling and) transferring the gastric digesta into simulated intestinal environments for further digestion using a shaking bath (Muir & O’Dea, 1992; Dean and Ma, 2007; Chen et al., 2014; Tagliazucchi et al., 2016), magnetic stirrer (De Boever et al., 2001; Al-Rabadi et al., 2009; Kazi and Tuzen, 2015), or inverting mixer (Oomen et al., 2003). Obviously, these approaches oversimplify the mixing patterns, and cannot reproduce the dynamic fluid behavior and the mechanical forces that chyme encounters in the GI tract resulting from contractions of stomach and intestinal walls (Kong and Singh, 2010). Moreover, the GI tract operates as a dynamic system, where digestion, emptying and absorption of nutrients occurs almost concurrently in a complex environment with different mixing and transport regimes.

Compared with a static model, a dynamic GI tract model is a better mimic of the physical processing and physiological events that occur in vivo as well as the effects of food properties (Moreno, 2007). Current trends in the development and utilization of dynamic in vitro digestion models for food applications have been summarized and reviewed elsewhere (Hur et al., 2010; Guerra et al., 2012; Minekus et al., 2014). The dynamic GI models such as dynamic gastric model (DGM) (Mercuri et al., 2011), TNO’s gastrointestinal model (TIM) (Minekus et al., 1995) and human gastric simulator (HGS) (Kong and Singh, 2010) have been developed to mimic the physical movements and chemical conditions present in the GI tract. Although, each model claims to have close simulation of in vivo conditions, these models largely ignored the geometric and morphological details of the real stomach or intestine which are thought to significantly affect digestive behaviors of food matrix within the GI tract (Chen et al., 2013). We here report the applicability of a
Dynamic *in vitro* Rat Stomach-Duodenum (DIVRSD) model that has mechanically similar geometrical and morphological details to the rat stomach and duodenum. This model is an upgraded version of the previous model, Dynamic *in vitro* Rat Stomach (DIVRS), which was validated by showing similar digestive behaviors of casein powder and large raw rice particles to that of *in vivo* conditions (rats), although the rate and extent of digestion and buffering ability were lower due to insufficient peristaltic contractions (Chen et al., 2013; Wu et al., 2014). The DIVRSD model is improved by incorporating a peristaltic (rolling) movement to the rat stomach model (together with the existing translational motion) and adding a duodenal model to make it more realistic compared to the DIVRS model previously reported.

To investigate applications of the DIVRSD model, three starch-based diets, namely control (low fiber), mango and pectin diets were passed through the model and investigated for their digestive behaviors (gastric emptying rate and hydrolysis of starch and protein) and rheological properties, and further characterized for microstructural changes of the duodenal digesta using microscopic techniques. The control diet is a starch based diet whereas in mango diets, 15% of mango powder as a source of fiber and phytochemicals is added to replace part of the wheat starch. Similarly, 11.8% of pectin was added as a source of soluble dietary fiber in the pectin diet. The composition and rheological properties of these diets and digesta from porcine stomach have been already reported (Wu et al., 2016). In addition, the effect of added porcine gastric mucin on rheology and simulated gastric emptying of the diets are investigated. The data reported demonstrate effects on the rate of starch and protein digestion as well as gastric emptying rate of diets containing various concentrations of soluble dietary fiber and gastric mucin using the DIVRSD model.

### 2. Materials and methods

#### 2.1. Materials

##### 2.1.1. Preparation of hydrated diets with various dry matter contents

Three kinds of diets namely control (low fibre), pectin and mango (for the composition of the diets, see Table 1 of Supplementary Material) were introduced into the DIVRSD model. The diets contained starch as the main energy source (65% in the control diet) with 18% protein from casein (13%), whey (3%) and egg powder (2%), and 4% fat from palm oil (3%) and sunflower oil (0.8%). Cellulose powder (6%) was added to all diets to prevent constipation. For the pectin diet, 11.8% of the starch was replaced by commercial pectin powder (equivalent to 10% pure pectin), and for the mango diet, 15% of the starch was replaced by commercial mango fruit powder. The diets were in powder form with measured dry matter contents of about
90% w/w. A sample of 5.0 g control, pectin and mango hydrated diets were mixed with deionized water followed by adjusting pH to 7.0 with 0.1 M HCl to achieve samples with different dry matter contents. Care was taken to avoid introduction of air bubbles through slow addition of water to dry diets, and all samples were allowed to rest for 15 mins prior to measurement in order to ensure complete hydration. The final dry matter contents for the control and mango diets were 20.35, 31.45 and 45.00%, whereas for the pectin diets, due to high viscosity hindering proper mixing, the maximum dry matter was limited to 25.00%. The samples were then incubated in a water bath at 37°C with constant stirring at 250 rpm for 30 min before in vitro digestion in the DIVRSD model.

2.1.2. Chemicals

In order to ensure the same ratio of enzymes and substrates, the amounts of enzymes added to artificial digestive juice were changed corresponding with the dry matter contents of the diets. So for higher dry matter content samples, a higher amount of enzyme was added. The composition of the three types of artificial digestive juice was as following. Rat saliva analogue was prepared with α-amylase (from Aspergillus oryzae, A9857, ≥150 units/mg protein) (19.90 mg, equivalent to 2985 units of α-amylase, per gram of dry sample), NaCl (0.117 mg/mL), KCl (0.149 mg/mL) and NaHCO₃ (2.1 mg/mL), and pH to 7.8 adjusted using 1 M NaOH (Kong and Singh, 2008a; Wu et al., 2014). Gastric juice was prepared with pepsin (from porcine gastric mucosa, P7000, ≥250 units/mg solid) (3.08 mg, equivalent to 770 units of protease, per gram of dry sample), NaHCO₃ (0.315 mg/mL), NaCl (8.775 mg/mL), with a pH of 1.6 adjusted using 1 M HCl (Kong and Singh, 2008a; Chen et al., 2013). Pancreatic and bile juice were prepared with pancreatin (from porcine pancreas, P-1750, 4 × USP specifications) (9.46 mg per gram of dry sample), bile salt (28.38 mg per gram of dry sample) and NaHCO₃ (4.5 mg/mL) adjusted pH of 7.50 using 1 M NaOH (Hutz et al., 1975; Pu et al., 2004). All the artificial digestive juice was kept in a water bath at 37°C for 10 min before use and were prepared fresh each day. The α-amylase, pepsin, pancreatin and bile salt were purchased from Sigma (Sigma-Aldrich, USA). p-hydroxybenzoic acid hydrazide (PAHBAH) Pierce BCA Protein Assay Kit (product No. 23225) used for protein analysis was provided by Thermo (Thermo Scientific, USA). All other chemicals were reagent grade and used as received.

2.2. Model development

The DIVRSD model is composed of a rat stomach device, a rat duodenum device, a secreting and emptying system and a temperature-controlled box (shown in Fig. 1A). The entire system rests on a large aluminum base plate. The rat stomach device is made up of a soft elastic rat stomach model created using a silicone
mold with the aid of an actual rat stomach with its inner-surface turned outwards, and an electric compression-rolling extrusion rig aimed to produce peristaltic contractions on the rat stomach wall. The procedures of making the soft rat stomach model and its geometric parameters have been reported previously (Chen et al., 2013; Wu et al., 2014). The completed silicone rat stomach has similar geometrical dimensions to the real rat stomach, with an approximate internal volume of 9.0 mL and occupying a box of around 4.0 cm by 3.0 cm by 2.5 cm. The electromechanical rig mainly consists of 2 stepper motors, 3 rollers, 3 eccentric wheels, 2 bevel gears, a belt, a driving shaft, and an angled compression plate. There are two different motions generated by the electromechanical rig, namely vertical compression on the part of fore-stomach imposed by the compression plate and rolling extrusion from the glandular portion toward the pylorus produced by two eccentric wheels. The frequency of these movements is dependent on the speed of the stepper motors, which are connected with a frequency controller, with adjustable speed of 0 to 10 rpm. The contraction amplitudes of compression and rolling extrusion can be set by changing the position of the compression plate and the gap between the two eccentric wheels, respectively. The mechanical force generated by the DIVRSD is in the range of 0.1 to 1.6 N determined using the method described elsewhere (Kong and Singh, 2010). During digestion trials, food materials are driven from the fore-stomach to the glandular portion due to the vertical compression (Fig. 1), while the rolling extrusion is responsible for breaking down large particles into small particulates as well as propelling the gastric contents from the glandular stomach into the pylorus and duodenum for further intestinal digestion. The gastric digesta is emptied through the “pressure pump” mechanism controlled by the opening size of a tapered tube (Fig. 1B) where the pressure gradient between the stomach and duodenum model is the main driving force. Also the tapered tube can provide a gastric sieving function such that the liquids and small particles (< 1~2 mm) are emptied from the stomach into the duodenum while the large particles are retained in the stomach for further disintegration. These physiological details make the rat stomach device similar to the in vivo system (DeSesso and Jacobson, 2001; Kong and Singh, 2008b).

The rat duodenum device consists of a silicon duodenum model that is made of a 15 cm long silicone tube with an inner diameter of 3.0 mm, and a mechanical driving instrument to produce peristaltic contractions. The duodenum model (shown in Fig. 1B) is linked with the pylorus through a T-shaped three-way union, whose other end is connected with a Y-shaped union for delivering pancreatic juice and bile juice. The driving instrument primarily consists of 4 eccentric wheels (placed in ‘S-shaped’ layout), 2 bevel gears, 2 belts, 2 stepper motors and a pulley system. It is applied to create segmented rolling extrusion on the duodenum model which is installed between the fixed pulley and eccentric wheel (Fig. 1B). The rolling
extrusion frequency ranges from 0 to 60 contractions per minute, which is also controlled by changing the speed of the stepper motors. The segmentations produced by the driving instrument are responsible for mixing the duodenal contents with digestive enzymes and bile juice, and propelling the contents to move forward along the duodenum (Lentle and Janssen, 2008; Bornhorst et al., 2013). The contraction amplitude of the rolling extrusion is in the range of 0 to 6 mm, which can be easily changed by adjusting the gap between the fixed pulley and eccentric wheel.

The secreting system is made up of three syringe pumps (TJP-3A/w0109-1B, Baoding Longer Precision Pump Inc., China), accounting for delivery of the artificial gastric juice into the rat stomach through the four secretion tubes, and pancreatic and bile juice into the duodenum through soft tubes connected with a Y-shaped union, respectively (shown in Fig. 1B). The flow rate of the syringe pumps can be adjusted between 0.000 and 10.000 mL/min. The temperature-controlled box made of acrylic plates is created to maintain the inner temperature around 37°C. The temperature is monitored by a thermocouple connected with an intelligent temperature controller.

2.3. In vitro batch digestion of hydrated diets

2.3.1. Collection of gastric and duodenal digesta

0.6 mL artificial gastric juice was injected into the rat stomach model before food loading to mimic the fasting state (Chen et al., 2013). 8.5 mL hydrated diet sample was mixed with 1.01 mL artificial saliva (37°C) and vortex mixed for 30 s (Wu et al., 2014) before being fed to the rat stomach model using a syringe. 0.2 mL mixture was immediately withdrawn (0 min) using a pipette for assay of reducing sugar (maltose) and protein. The electric compression - roll extrusion rig was set to create 3 contractions per minute and the amplitude of the angled plate was set at 2.6 mm (Chen et al., 2013). The artificial gastric juice was then fed continuously at controlled rates. The gastric secretion rate was changed with digestion time with a mean value of 25 µL/min over 180 min digestion through the secretion tubes using a syringe pump, with values based on previous comparisons with in vivo data (Chen et al., 2013). Meanwhile, the mechanical driving instrument of the duodenum device was set to produce 36 contractions per minute (Lentle et al., 2012) and the secreting rates of artificial pancreatic juice and bile juice were set at 30 µL/min (Hotz et al., 1975; Ofem et al., 2013) using the other two syringe pumps. The food materials were digested in the DIVRSD model in separate batches for 10, 20, 30, 40, 60, 90, 120 and 180 min. After the designated digestion time, the DIVRSD was stopped and the contents remaining in the stomach (gastric digesta) and the duodenum (duodenal digesta) were both collected and their volumes measured using a graduated centrifuge tube.
Similar procedures were carried out for all the other batches of samples after being digested for the specified time in order to collect the gastric and duodenal digesta for analyses of pH, starch and protein hydrolysis, rheological and microstructural properties.

2.3.2. Determination of pH, starch and protein hydrolysis (%)

The pH of the gastric and duodenal digesta was determined immediately after collection following mixing to ensure homogeneity. The maltose concentration was measured using the p-hydroxybenzoic acid hydrazide (PAHBAH) method (Moretti and Thorson, 2008). A factor of 0.95 was used to convert reducing sugar (maltose) values to starch content. The protein concentration in the supernatant of the gastric and duodenal digesta was measured using the Pierce BCA Protein Assay Kit (product No. 23225, purchased from Thermo Scientific) according to the manufacturer’s protocol using bovine serum albumin (BSA) as a reference standard. The starch and protein hydrolysis (%) as a result of the combined gastric and duodenal phases was thus calculated based on the maltose / protein concentration and volume of the gastric and duodenal digesta. The specific procedures for determining the maltose and protein concentration as well as calculation of the starch / protein hydrolysis are shown in Supplementary Material.

2.3.3. Rheological measurements

Due to limitations in the amount of gastric digesta remaining at the end of digestion and the limited duodenal digesta available at the initial digestion stages, digesta collected after 30, 60 and 120 min digestion were selected for rheological measurements. The steady shear and dynamic oscillatory tests were conducted with a stress-controlled rheometer (AR-G2, TA Instruments, New Castle, DE, USA) using a parallel plate geometry (40 mm diameter) at a gap of 500 µm. The sample (1.0 mL) was subjected to a strain sweep to obtain the linear viscoelastic regime followed by oscillatory measurements over the frequency range of 0.1-100 rad/s. The steady shear measurements were performed for shear rates ranging from 0.1 to 100 1/s. Normal forces were effectively constant during measurement and there was no evidence of slip effects. All measurements were performed at 37°C and were carried out in triplicate.

2.3.4. Microstructural characterization of duodenal digesta

Microstructural examinations were carried out to characterize the microstructure of the duodenal digesta of the control, pectin and mango diets after digestion in the DIVRSD model for 0, 30 and 120 min using a Scanning Electron Microscope (SEM) as described by Dhital et al. (2014).
2.4. Effect of porcine gastric mucin on in vitro digestion of hydrated diets

In order to investigate the effect of gastric mucin on in vitro digestion of the control, pectin and mango diets in the DIVRSD model, porcine gastric mucin solutions with concentrations of 0, 10 and 20 mg/mL were mixed thoroughly with the control, pectin and mango diets to achieve a dry matter content of 20.35% before being injected into the soft rat stomach model. The gastric emptying rate, and starch and protein hydrolysis extent of the samples were determined throughout the 180 min digestion (after 10, 20, 30, 40, 60, 90, 120 and 180 min) as described in section 2.3. Rheological properties were also measured after 30 min digestion time.

2.5. Statistical analysis

Statistical analysis was conducted using a three-way analysis of variance (ANOVA) in the GLM procedure of the SAS system to analyze how the interactions among diet variety, dry matter content and digestion time affected digesta rheology (power law parameters K and n), starch and protein hydrolysis (hydrolysis extent after 180 digestion) as well as gastric emptying rates (half time $t_{1/2}$, and lag phase time, $t_{lag}$) of the diets when subjected to the DIVRSD model. Two-way ANOVA was performed to investigate the effects of diet variety and dry matter contents on digesta pH at the end of digestion ($t=180$ min). The effects of porcine gastric mucin at the concentrations of 0, 10 and 20 mg/mL on the duodenal digesta rheology (after 30 min digestion) and gastric emptying rate of the diets at the dry matter content of 20.35% were also compared using two-way (including diet variety and porcine gastric mucin concentration) ANOVA. Statistical significance was set at a probability level of 0.05. All results were expressed as means ± standard deviations. Each trial for collection of the gastric and duodenal digesta of the same diet was replicated 3 times to ensure good reproducibility.

3. Results and discussion

3.1. pH of the gastric and duodenal digesta

Fig. 2A shows the changes in pH of the gastric and duodenal digesta of the control, pectin and mango diets with various dry matter contents (%). It can be seen the pH values of the gastric digesta regardless of diet varieties and dry matter contents were increased remarkably from the initial value of 1.6 to almost neutral pH when the food materials were fed to the rat stomach model. This is due to the diluting effect of feed injected into the system. However, they were gradually decreased in the later digestion time due to the continuous secretion of gastric juice, and further emptying of the gastric contents leading to reduced
buffering ability. In contrast to the gastric digesta, a slight increase in the pH of the duodenal digesta was observed mainly due to the addition of bile juice and pancreatic juice (duodenum), but no significant difference was observed among the diets (p>0.05). These trends are consistent with the previous in vivo results conducted on living rats (Chen et al., 2013; Wu et al., 2014). Further, the final gastric digesta pH of the diets with the highest dry matter content (i.e. 45% for the control and mango diets) was significantly higher than those with the lowest dry matter content (20.35%) (p<0.05), suggesting that the buffering ability of the food materials could be increased with the increase of the solid content. It is interesting to note that the final pH of the gastric digesta of the pectin diet (pH 3.7) was significantly higher than those of the control (pH 2.5) and mango (pH 2.4) digesta at the same concentration of 20.35% (p<0.05), possibly reflecting the greater viscosity and modulus of the pectin digesta as shown in the next section.

3.2. Gastric emptying of the control, pectin and mango diets

Gastric emptying is a complex phenomenon, not only relating to the gastro-duodenal pressure gradient and mechanical force imposed on the stomach, but also the structure and physicochemical properties of the food matrix such as particle size, viscosity and food volume (Kong and Singh, 2008b). In this study, gastric emptying was expressed by the fraction of meal retention in the stomach (%) respect to digestion time, which was calculated by dividing the volume of digesta remaining in the stomach by the total volume of materials (including food materials and gastric juice) introduced into the stomach at given time points. Fig. 2B shows the fractional retention in the stomach of the three diets after in vitro batch digestion in the DIVRSD model for different times. All the diets presented a consistent trend with continuous decrease in the fractional retention in the stomach with digestion time, however, a lag phase was observed for the diets with the highest dry matter content (45% for the control and mango diets, 25% for the pectin diet), that is, with an initial delay followed by rapid emptying. For the control and mango diets with the lowest dry matter content (20.35%), emptying began instantly upon ingestion and proportionally to the volume of digesta remaining in the stomach. The different gastric emptying behaviors of the diets with different dry matter contents were also reported by Camilleri et al. (1985), who found a lag phase of the gastric emptying curve for particle-dominated meals while no such lag phase for liquid meals in healthy volunteers. Similar results were reported by Kong and Singh (2010) that the mass retention ratio of raw carrots had a sigmoidal decrease with time (close to solid emptying curve), while carrots cooked for 6 min had an exponential decay (close to emptying for liquids).

A modified Elashoff’s model was proposed for evaluation of gastric emptying rate, as indicated by fraction
of food retention compared with time (Siegel et al., 1985):

\[ y_t = 1 - (1 - e^{-kt})^\beta \]  

(1)

where \( y_t \) is the fractional meal retention at time \( t \) in minutes, \( k \) is the gastric emptying rate per minute, and \( \beta \) is the extrapolated y-intercept from the terminal portion of the curve. A value of \( \beta > 1.0 \) indicates an initial delay in emptying as for the solid foods, whereas a value of \( \beta < 1.0 \) indicates an initial rapid emptying as for liquid foods.

The half-time \( (t_{1/2}) \), the time required for the initial food to be reduced by half, can be calculated using \( y_t = 0.5 \) and solving for \( t \):

\[ t_{1/2} = \left( -\frac{1}{k} \right) \times ln(1 - 0.5^{\frac{1}{\beta}}) \]  

(2)

Lag phase time \( (t_{lag}) \) can be calculated from when the 2nd derivative of the modified Elashoff’s function (1) is equal to zero:

\[ t_{lag} = \frac{\ln \beta}{k} \]  

(3)

The parameters of the modified Elashoff’s model for the diets with various dry matter contents and statistical results are summarized in Table 1. It can be seen that the values of half time \( (t_{1/2}) \) and lag phase time \( (t_{lag}) \) both significantly increase with the increasing dry matter content within each diet, indicating that gastric emptying rate was decreased with the increase of solids content. The values of \( \beta \) are all greater than 1 except for the control and mango diet with the dry matter content of 20.35% suggesting most of the diets were showing some solid-like behavior with an initial delay (lag phase) in gastric emptying (Siegel et al., 1985; Kong and Singh, 2008b, 2010). There is no significant difference in these parameters (including \( \beta \), \( t_{1/2} \) and \( t_{lag} \)) between the control and mango diet at the same dry matter content (p>0.05), however, the parameters such as \( t_{1/2} \) and \( t_{lag} \) are significantly greater for the pectin diet compared with the control or mango diet (p<0.05). This could be due to the viscous property of pectin that hindered the free movement and mixing of digesta during digestion in the rat stomach thus delaying the gastric emptying rate (Lentle and Janssen, 2008; Wu et al., 2016). Additionally, the values of \( r^2 \) are close to 1 indicating a good fit of the modified Elashoff’s model.

3.3. Rheological properties of the gastric and duodenal digesta

It has been reported that rheological properties of ingested diets change over time along the GI tract due to the breakdown and emptying of food particles as well as the secretions of digestive juices (Bornhorst et al., 2013). Fig. 3A shows the mean (n=3) storage modulus (G’) and loss modulus (G”) of the gastric and duodenal digesta of the control, pectin and mango diets with the dry matter content of 20.35% after
digestion in the DIVRSD model for 30, 60 and 120 min, respectively. The moduli of digesta from the diets with other dry matter contents are shown in Fig. S1A and Fig. S1B of Supplementary Material. As shown for the control and mango diets at 20.35%, the gastric and duodenal digesta both showed a dilute solution or more liquid-like behavior with \( G'' \) generally higher than \( G' \) over 120 min digestion; when the dry matter increased to 31.45%, both of the digesta collected after 30 and 60 min digestion showed weak gel or more solid-like behavior with \( G' \) greater than \( G'' \), while they still showed a dilute solution behavior after 120 min digestion; at the dry matter content of 45%, they presented weak gels after 30 and 60 min digestion over the entire frequency range, however, they changed to concentrated solution or entanglement network system after 120 min digestion, with the curves of \( G' \) and \( G'' \) intersecting in the middle of the frequency range (Ross-Murphy et al., 1983; Ndjouenkeu et al., 1995). Different from the control and mango diets, the gastric digesta of the pectin diet with the lowest dry matter (15.15%) showed concentrated solution behavior at initial digestion (t=30 min) followed by dilute solution or more liquid-like behavior as time progressed, while the duodenal digesta always showed dilute solution behavior over the whole digestion time. With the increase of dry matter content, the digesta changed from liquid-like to weak gel behavior, as higher concentrations of pectin contribute to the formation of more stable elastic solid network system (Xu et al., 2008). In addition, for all the digesta, the higher the dry matter content, the less the frequency dependence of \( G' \) and \( G'' \), indicating that the stability of temporary networks was enhanced with the increase of solid content (Xu et al., 2008).

The mean (n=3) dynamic and steady shear viscosity of the gastric and duodenal digesta of the control, pectin and mango diet (20.35%) are shown in Fig. 3B and the cases of the diets with other dry matter contents are presented in Fig. S2A and Fig. S2B of Supplementary Material. It can be seen that the dynamic viscosity and steady shear viscosity of the digesta both decreased with the increase of frequency and shear rate respectively, irrespective of dry matter and diet variety, indicating the non-Newtonian flow behaviors of all the tested systems (Ross-Murphy et al., 1983). Furthermore, for all the diets at the same dry matter content (i.e. 20.35%) and digestion time, the dynamic viscosity was always higher than the steady shear viscosity over the whole frequency or shear rate ranges studied. This revealed that a yielding process occurred as the digesta started to move under large deformation conditions with a structural breakdown at the initiation of flow (Takahashi and Sakata, 2002, 2005; Wu et al., 2016).

The non-Newtonian flow behavior under steady shear measurements is generally fitted with the power law model (Holdsworth, 1971) expressed as follows:
where \( \eta \) = steady shear viscosity or apparent viscosity, \( Pa \cdot s \), \( K \) = consistency constant, \( Pa \cdot s^n \), \( \dot{\gamma} \) = shear rate, \( 1/s \), and \( n \) = power law index or flow behavior index. Table 2 shows the power law parameters for the steady shear viscosity of the gastric and duodenal digesta. It can be seen that the values of power law indices (n) under steady shear measurements are all between 0 and 1 indicating shear-thinning behavior of the digesta with reduction of the digesta viscosity with increase in shear rate (Ross-Murphy et al., 1983). It should be noted that this model is fitted to data from samples which have already undergone a yielding process (as indicated by the greater value of dynamic compared with steady shear viscosity). For each diet, the steady shear viscosity of the digesta was less dependent on shear rate with n generally higher at the lower dry matter content compared to the higher dry matter content (Table 2), although the differences are not always statistically significant. This might suggest a greater shear-thinning behavior of the diets with higher dry matter contents. The values of \( K \) for all digesta significantly increased with the increasing dry matter content indicating that the digesta viscosity was enhanced significantly with the increase of dry matter content. Further, as the digestion time progressed from 30 min to 120 min, the values of \( K \) decreased significantly (Table 2) for all the diets due to the dilution effect of digestive juice on the digesta viscosity. In addition, in most situations, the viscosity of the gastric digesta was higher than the duodenal digesta (Table 2) mainly because of further dilution in the duodenum due to the continuous secretions of bile and pancreatic juice as well as starch and protein hydrolysis. Irrespective of the different increase in dry matter content from 20.35% to 45% for control and mango diets and from 15.15% to 25% for the pectin diet, the \( K \) increased more than 10 times, which is in line with the results reported by McRorie et al. (2000) who found that apparent viscosity increased 45-fold when the dry matter content of porcine digesta increased from 12.5% to 28.5% between the caecum and the rectum. This indicated that viscosity of the digesta at higher dry matter content may be largely governed by particle-particle interactions rather than hydrodynamic effects in which the streamlines of the liquid phase diverge around each particle during flow (Barnes et al., 1989). The particle-particle interactions among the digesta are believed to significantly influence the processes of digestion that depend on flow and mixing within the GI tract (Lentle and Janssen, 2008).

Compared with the control and mango diets that had no significant difference in the digesta viscosity (as represented by \( K \)), however, the viscosity of the pectin diet even with lower dry matter content was significantly higher than those of the control or mango diet with higher dry matter content (Table 2) due to the viscous property and high water binding ability of the pectin that hindered the movement and mixing of the digesta in the GI tract (Takahashi and Sakata, 2005; Lentle and Janssen, 2008). Similar results were
reported by Takahashi et al. (2009) who investigated the effect of insoluble fibers with different water-holding capacity on the viscosity of gastric, small intestinal and caecal contents in rats and concluded that the digesta viscosity was significantly elevated by insoluble fibers with higher water-holding ability. *In vivo*, the increased GI rheological properties and decreased mixing efficiency could lead to reduced mass transfer across the intestinal wall, resulting in lower amounts of nutrient absorption (Bornhorst et al., 2013).

### 3.4. Starch and protein hydrolysis (%)

Fig. 4 presents starch and protein hydrolysis (%) of the control, pectin and mango diets with various dry matter contents as time progressed. Similar digestive behavior was found for all the diets independent of dry matter with rapid starch and protein hydrolysis upon initial digestion followed by an approach to a plateau. This is in line with expectations of the usual first order kinetics of both starch and protein hydrolysis (Butterworth et al., 2012), but in this case there are additional factors that may play a role which are not the same for starch and protein hydrolysis. The initial rapid increase of starch hydrolysis could in part be due to the increased action of α-amylase on starch molecules in the fore-stomach of the rat stomach model. The subsequent activity of α-amylase in the stomach model could be inhibited due to the continuous secretion of gastric juice, leading to a lower rate of starch hydrolysis at the end of digestion. As for protein hydrolysis, the initial higher rate of protein hydrolysis could also be in part due to the increased action of pepsin on protein. However, as the volume of food material remaining in the stomach decreased due to gastric emptying, the protein available for further digestion was reduced and the neutral pH duodenal environment inhibited gastric pepsin. As a result, the protein hydrolysis rate was decreased in the end. The digestive trends of starch and protein were in accordance with the results obtained from *in vitro / in vivo* digestion of cooked rice starch and casein powder, respectively (Chen et al., 2012, 2013).

It can also be seen that the diets with lower dry matter content showed a higher extent of starch and protein hydrolysis compared to those with higher dry matter content (Fig. 4). This is mainly associated with the increasing rheological properties such as steady shear viscosity of the gastric and duodenal digesta with increasing dry matter. Higher viscosity component not only slows down gastric emptying rate (Fig. 2B) and retards the mixing and flow along the GI tract, but also reduces the random diffusion of enzymes that can lead to a reduction in probability of enzyme binding to substrate thus lowering the rate and extent of hydrolysis of substrate (Dhital et al., 2014). Compared with the control and mango diet which show no significant differences in starch and protein hydrolysis (p>0.05), their digestion in the pectin diet was significantly lower (p<0.05). For example, for the diets with the same dry matter content of 20.35%, the
starch hydrolysis of the control and mango diets was almost the same (~32%), while the pectin diet was only ~22% digested in the end. The lower extent of starch and protein digestion of the pectin diet was probably due to the highly viscous property of the pectin that greatly increased the digesta viscosity (Kong and Singh, 2008b; Lentle and Janssen, 2008).

3.5. Microstructural properties of the duodenal digesta

SEM images of the duodenal digesta of the control, pectin and mango diets (20.35%) obtained from 0, 30 and 120 min digestion in the DIVRSD model are presented in Fig. 5. The starch granules of the control and mango digesta showed similar microstructure and morphology at the same digestion time, which however were different from those of the pectin digesta. Specifically, at the initial stage of digestion (t=0 min) for the control and mango diets, the starch granules mostly exhibited regular, intact and oval-like shape with relatively smooth surfaces. After 30 min of hydrolysis (Fig. 5A-30 min and C-30 min), deformations were observed in some granules with slight exo-erosion and rough structure on the surface, and adhesion between some of the granules was also detected. This pattern became more noticeable after 120 min digestion (Fig. 5A-120 min and C-120 min) with a number of starch granules aggregating with each other and more granules showing progressive surface erosion. Furthermore, several starch granules were observed as pitted, with some small pores or holes that were randomly distributed on the surface, due to the action of α-amylase present in the artificial digestive juice. However, the starch granules in the pectin digesta were mostly bonded together and trapped in the pectin network structure or covered by the pectin gel particularly at the early stages of digestion (Fig. 5B-30 min). This could impede the contact of the starch and protein granules with α-amylase and pepsin thus preventing starch and protein digestion (Takahashi and Sakata, 2005). As digestion time progressed and with continuous dilution of the digestive juice, the pectin gel structures disappeared gradually and some of the granules became visible, but there were still no distinct pores on the surface after 120 min digestion. The lower extent and rate of starch and protein hydrolysis (Fig. 4) as well as gastric emptying (Fig. 2B) of the pectin diet compared with the control and mango diets may result from the viscous property of pectin that increased the digesta viscosity thus hindering the flow and mixing with the digestive enzymes. Similar microstructural differences between the pectin diet and control or mango diet during digestion were also observed in the previous in vivo study on pigs (Wu et al., 2016).

3.6. Effect of porcine gastric mucin on in vitro digestion of the diets

The rheological properties of the duodenal digesta (t=30 min) of the three diets after addition of 0, 10 and 20 mg/mL of the porcine gastric mucin are shown in Fig. 6. Table 3 presents the power law parameters for the
The steady shear viscosity of the duodenal digesta. The values of $K$ for the control and mango diets are significantly increased with increasing concentration of added mucin ($p<0.05$), indicating that gastric mucin could significantly enhance digesta viscosity for these diets. As shown in Fig. 6, for the digesta of the control and mango diets without addition of the gastric mucin, the values of $G'$ were lower than $G''$ over the whole frequency indicating a liquid-like behavior of the digesta, however, after addition of more than 10 mg/mL gastric mucin to the diets, the digesta changed to solid-like or weak gel behavior with $G'$ always higher than $G''$. Compared with the control and mango digesta, the viscosity of the pectin digesta was also significantly increased with the $K$ increased from 6.67 to 15.40 Pa·s after addition of 20 mg/mL of mucin ($p<0.05$). Nonetheless, it was not significantly increased when 10 mg/mL of the mucin was added ($p>0.05$) and the pectin digesta always showed solid-like behavior, which could be due to the highly viscous property of pectin playing a dominant role in the rheology compared with the gastric mucin. These findings were in agreement with previous results where we reported the effect of gastric mucin on rheological parameters of porcine feeds and gastric digesta (Wu et al., 2016).

Fig. 7 shows the gastric emptying as well as starch and protein hydrolysis of the pectin diet (20.35%) in the presence of 0, 10 and 20 mg/mL of the gastric mucin during digestion in the DIVRSD model. The cases of the control and mango diets are shown in Fig. S3A and Fig. S3B of Supplementary Material. It can be seen that the gastric emptying rates of the three diets were significantly reduced by the mucin, with the half time ($t_{1/2}$) and lag time ($t_{lag}$) increased significantly with the increase of the gastric mucin added to the diets (Table 4). The values of $\beta$ are greater than 1 indicating that the control and mango diets changed from a liquid-like system to more solid-like system after addition of the gastric mucin, which was in line with the rheological results (Fig. 6 and Table 3). As might be expected, the starch and protein hydrolysis of the diets were also decreased in the presence of the gastric mucin and the pectin diet experienced the largest decline due to the combined effects of the pectin and mucin (Fig. 7).

It should be noted that the levels of porcine gastric mucin used in this study were based on the previous results that up to 20 mg/mL porcine gastric mucin was needed to match the rheology of porcine gastric digesta samples (Wu et al. 2016). Although this concentration of mucin is higher than expected in vivo, commercial mucin is degraded compared with native mucin and therefore needs to be added at higher levels to obtain an equivalent rheology (Celli et al., 2007). The role of the gastric mucin contributing to the increased viscoelastic properties and the reduced rates of digestion and gastric emptying is probably associated with the mucin-mucin and mucin-protein interactions that caused aggregation and gelation in
acidic environment (Bansil and Turner, 2006; Celli et al., 2007). These interactions would be expected to reduce the biochemical reaction rate by increasing digesta viscosity leading to lower flow and mixing efficiency as well as by preventing the effective contact of starch and protein with enzymes (Lentle and Janssen, 2008). Thus, the effect of gastric mucin on rheology, digestion as well as gastric emptying rates should not be neglected when simulating food digestion and absorption.

4. Conclusion

We studied the fate of starch and protein and the effect of porcine gastric mucin on digesta rheology and gastric emptying rate as well as hydrolysis of macronutrients in control, pectin and mango diets with various dry matter contents using an improved DIVRSD model. The model, due to incorporation of a rolling-extrusion movement on the silicone stomach wall together with a dynamic duodenum model, is a better mimic of physiological and biochemical conditions presented in the rat stomach and duodenum. The results obtained from the DIVRSD indicated that it was able to provide a reasonably realistic set of conditions that mimic the processes of digestion and gastric emptying of the pectin and mango enriched diets, emphasizing the significance of developing good in vitro digestion models for studying food digestion processes within the GI tract. Starch and protein hydrolysis decreased with the increasing dry matter contents due to increased digesta viscosity. The gastric emptying rate as expressed by the fractional retention ratio in stomach was decreased with the increasing dry matter content of the diets, and the diets with higher dry matter content were emptied with an initial delay in emptying indicating solid-like emptying behavior, while the lower dry matter diets were emptied with an initial rapid emptying similar to the emptying of liquids. The rheological properties such as dynamic and steady shear viscosity of the gastric and duodenal digesta were significantly decreased with increasing digestion time due to the dilution effect from continuous secretion of digestive juice and hydrolysis of starch and protein. There is no distinct difference in the rates of gastric emptying or starch and protein hydrolysis between the control and mango diet, however, they are significantly decreased for the pectin diet due to the highly viscous property of pectin that would hinder flow and mixing. SEM images revealed that the starch granules and protein particles were trapped in the pectin gel network, which could be responsible for the lower rates of starch and protein hydrolysis as well as gastric emptying of the pectin diet than that of the control or mango diet by hindering the contact of the substrates with digestive enzymes. Addition of porcine gastric mucin to the feeding diets could markedly increase the digesta rheology leading to lower digestion and gastric emptying rates.
Acknowledgements

The China Scholarship Council (CSC) is thanked for providing the scholarship for the first author to study abroad at The University of Queensland. This work was supported by the Australian Research Council Centre of Excellence in Plant Cell Walls CE110001007.

References


Tables

Table 1

Modified Elashoff’s model parameters for gastric emptying of the hydrated diets with various dry matter contents\(^1, 2\)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Dry matter (%)</th>
<th>(k) (\pm) SD</th>
<th>(\beta) (\pm) SD</th>
<th>(t_{1/2}) (min) (\pm) SD</th>
<th>(t_{lag}) (min) (\pm) SD</th>
<th>(r^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20.35</td>
<td>0.048 ± 0.008(^a)</td>
<td>0.964 ± 0.011(^d)</td>
<td>13.96 ± 1.68(^d)</td>
<td>0(^d)</td>
<td>0.996</td>
</tr>
<tr>
<td></td>
<td>31.45</td>
<td>0.031 ± 0.005(^b)</td>
<td>1.143 ± 0.038(^c, d)</td>
<td>25.55 ± 2.02(^c)</td>
<td>2.78 ± 0.65(^c)</td>
<td>0.997</td>
</tr>
<tr>
<td></td>
<td>45.00</td>
<td>0.022 ± 0.003(^c)</td>
<td>1.743 ± 0.133(^b)</td>
<td>50.84 ± 2.55(^b)</td>
<td>17.92 ± 1.68(^b)</td>
<td>0.993</td>
</tr>
<tr>
<td></td>
<td>15.35</td>
<td>0.026 ± 0.006(^k, c)</td>
<td>1.187 ± 0.098(^b)</td>
<td>30.96 ± 1.98(^c)</td>
<td>7.79 ± 0.87(^c)</td>
<td>0.995</td>
</tr>
<tr>
<td>Pectin</td>
<td>20.35</td>
<td>0.024 ± 0.004(^c)</td>
<td>1.910 ± 0.201(^a, b)</td>
<td>49.56 ± 3.02(^b)</td>
<td>24.89 ± 1.45(^b)</td>
<td>0.995</td>
</tr>
<tr>
<td></td>
<td>25.00</td>
<td>0.019 ± 0.003(^c)</td>
<td>2.138 ± 0.211(^d)</td>
<td>68.82 ± 3.55(^a)</td>
<td>31.66 ± 2.18(^a)</td>
<td>0.997</td>
</tr>
<tr>
<td></td>
<td>20.35</td>
<td>0.035 ± 0.002(^b)</td>
<td>0.980 ± 0.006(^b)</td>
<td>19.20 ± 1.66(^d)</td>
<td>0(^d)</td>
<td>0.998</td>
</tr>
<tr>
<td>Mango</td>
<td>31.45</td>
<td>0.029 ± 0.002(^b, c)</td>
<td>1.126 ± 0.022(^b, d)</td>
<td>26.39 ± 2.18(^c)</td>
<td>3.39 ± 0.46(^c)</td>
<td>0.995</td>
</tr>
<tr>
<td></td>
<td>45.00</td>
<td>0.020 ± 0.001(^c)</td>
<td>1.605 ± 0.120(^b)</td>
<td>53.49 ± 2.79(^a, b)</td>
<td>26.31 ± 1.15(^b)</td>
<td>0.995</td>
</tr>
</tbody>
</table>

\(^1\)Values represent the mean ± standard deviation of triplicate tests.

\(^2\)Values in the same column for the model parameters followed by different superscripts are significantly different (p <0.05).

It should be noted that the response variables (including \(k\), \(\beta\), \(t_{1/2}\) and \(t_{lag}\)) were statistically influenced only by the dry matter content for the same diet and the diet variety at the same dry matter content, so different diets with different dry matter contents should not be directly compared.
Table 2

Power law parameters for the steady shear viscosity of the gastric and duodenal digesta of the hydrated diets with various dry matter contents.\(^1,2\)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Dry matter (%)</th>
<th>Time (min)</th>
<th>Stomach</th>
<th>Duodenum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(K) (Pa(\cdot)s(^n))</td>
<td>(n)</td>
</tr>
<tr>
<td>20.35</td>
<td></td>
<td>30</td>
<td>1.531 ± 0.232(^{c, g})</td>
<td>0.324 ± 0.046(^f)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>0.445 ± 0.116(^b)</td>
<td>0.420 ± 0.031(^{d, e})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>0.093 ± 0.032(^d)</td>
<td>0.727 ± 0.112(^g)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>6.267 ± 0.786(^d)</td>
<td>0.401 ± 0.008(^{d, e})</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>31.45</td>
<td>1.674 ± 0.322(^{c, g})</td>
<td>0.380 ± 0.076(^c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>0.153 ± 0.032(^d)</td>
<td>0.728 ± 0.055(^c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>9.892 ± 0.895(^c)</td>
<td>0.104 ± 0.004(^{j})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45.00</td>
<td>4.850 ± 0.466(^e)</td>
<td>0.145 ± 0.039(^{b, i})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>0.599 ± 0.177(^b)</td>
<td>0.545 ± 0.049(^c)</td>
</tr>
<tr>
<td>Pectin</td>
<td></td>
<td>20.35</td>
<td>1.433 ± 0.155(^f)</td>
<td>0.117 ± 0.033(^{j})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>0.486 ± 0.103(^b)</td>
<td>0.305 ± 0.019(^{g, k})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>0.196 ± 0.064(^d)</td>
<td>0.259 ± 0.047(^f)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>11.45 ± 0.69(^{b, c})</td>
<td>0.344 ± 0.073(^{f, r})</td>
</tr>
<tr>
<td></td>
<td>25.00</td>
<td>60</td>
<td>1.992 ± 0.220(^{g})</td>
<td>0.326 ± 0.030(^f)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>0.545 ± 0.113(^b)</td>
<td>0.532 ± 0.089(^c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>0.358 ± 0.65(^{d, e})</td>
<td>0.497 ± 0.055(^{b, e})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25.00</td>
<td>12.10 ± 0.93(^b)</td>
<td>0.222 ± 0.032(^{g, h})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>2.763 ± 0.232(^c)</td>
<td>0.341 ± 0.076(^{f, r})</td>
</tr>
<tr>
<td>Mango</td>
<td>31.45</td>
<td>60</td>
<td>1.811 ± 0.144(^{g})</td>
<td>0.380 ± 0.080(^{j})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20.35</td>
<td>0.398 ± 0.035(^b)</td>
<td>0.216 ± 0.007(^{g, h})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>0.085 ± 0.027(^d)</td>
<td>0.612 ± 0.082(^b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>6.287 ± 0.588(^{d, e})</td>
<td>0.402 ± 0.055(^{b, e})</td>
</tr>
<tr>
<td></td>
<td>45.00</td>
<td>60</td>
<td>6.558 ± 0.254(^d)</td>
<td>0.125 ± 0.008(^{i})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>0.518 ± 0.102(^b)</td>
<td>0.443 ± 0.089(^{d})</td>
</tr>
</tbody>
</table>

\(^1\)Values represent the mean ± standard deviation of triplicate tests.

\(^2\)Values in the same column for the model parameters followed by different superscripts are significantly different (\(p < 0.05\)).

It should be noted that the response variables \(K\) and \(n\) were statistically influenced only by the dry matter content (for the
same diet and digestion time), the diet type (for the same dry matter content and digestion time) and the digestion time (for the same diet and dry matter content), so different diets with different dry matter contents or at different digestion times should not be directly compared.

Table 3

<table>
<thead>
<tr>
<th>Diet</th>
<th>Mucin (mg/mL)</th>
<th>K (Pa·s^n)</th>
<th>n</th>
<th>r^2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0.667 ± 0.042^b</td>
<td>0.323 ± 0.038^a</td>
<td>0.985</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2.387 ± 0.050^d</td>
<td>0.325 ± 0.033^a</td>
<td>0.997</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>4.343 ± 0.147^c</td>
<td>0.426 ± 0.018^b</td>
<td>0.993</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>6.674 ± 0.248^b</td>
<td>0.248 ± 0.065^c</td>
<td>0.999</td>
</tr>
<tr>
<td>Pectin</td>
<td>10</td>
<td>7.952 ± 0.376^b</td>
<td>0.247 ± 0.045^c</td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>15.40 ± 0.89^a</td>
<td>0.238 ± 0.055^c,d</td>
<td>0.998</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0.578 ± 0.077^c</td>
<td>0.182 ± 0.067^d</td>
<td>0.999</td>
</tr>
<tr>
<td>Mango</td>
<td>10</td>
<td>2.216 ± 0.287^d</td>
<td>0.233 ± 0.045^c,d</td>
<td>0.993</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>4.282 ± 0.465^c</td>
<td>0.303 ± 0.077^h,c</td>
<td>0.995</td>
</tr>
</tbody>
</table>

1Values represent the mean ± standard deviation of triplicate tests.

2Values in the same column for the parameters followed by different superscripts are significantly different (p < 0.05). It should be noted that the response variables (K and n) were statistically influenced only by the diet variety (at the same mucin concentration) and the mucin concentration (within the same diet), so different diets with addition of different mucin concentrations should not be compared directly.
Table 4

Modified Elashoff’s model parameters for gastric emptying of the hydrated diets with the same dry matter content of 20.35% after addition of 0, 10 and 20 mg/mL porcine gastric mucin.\(^1,2\)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Mucin (mg/mL)</th>
<th>(k) (Values ± Standard Deviation)</th>
<th>(\beta) (Values ± Standard Deviation)</th>
<th>(t_{1/2}) (Values ± Standard Deviation)</th>
<th>(t_{lag}) (Values ± Standard Deviation)</th>
<th>(r^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0.048 ± 0.006(^a)</td>
<td>0.964 ± 0.008(^d)</td>
<td>13.96 ± 0.54(^e)</td>
<td>0(^f)</td>
<td>0.996</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.040 ± 0.002(^b)</td>
<td>1.036 ± 0.032(^d)</td>
<td>17.12 ± 1.05(^e)</td>
<td>0.74 ± 0.27(^d)</td>
<td>0.988</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.028 ± 0.002(^c)</td>
<td>1.256 ± 0.012(^c)</td>
<td>30.82 ± 1.77(^f)</td>
<td>5.70 ± 0.76(^c)</td>
<td>0.993</td>
</tr>
<tr>
<td>Pectin</td>
<td>0</td>
<td>0.024 ± 0.003(^c,d)</td>
<td>1.910 ± 0.059(^d)</td>
<td>49.06 ± 2.76(^b)</td>
<td>23.11 ± 1.45(^b)</td>
<td>0.995</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.022 ± 0.001(^d)</td>
<td>2.146 ± 0.144(^b)</td>
<td>59.67 ± 3.12(^a)</td>
<td>31.82 ± 2.02(^a)</td>
<td>0.995</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.020 ± 0.002(^d)</td>
<td>2.229 ± 0.177(^b)</td>
<td>67.95 ± 2.88(^a)</td>
<td>36.43 ± 2.45(^a)</td>
<td>0.993</td>
</tr>
<tr>
<td>Mango</td>
<td>0</td>
<td>0.035 ± 0.004(^b)</td>
<td>0.980 ± 0.006(^d)</td>
<td>19.20 ± 0.77(^e)</td>
<td>0(^f)</td>
<td>0.998</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.031 ± 0.004(^c)</td>
<td>1.058 ± 0.010(^d)</td>
<td>23.43 ± 1.11(^d)</td>
<td>1.61 ± 0.48(^d)</td>
<td>0.989</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.026 ± 0.003(^c,d)</td>
<td>1.197 ± 0.023(^c,d)</td>
<td>32.11 ± 1.45(^e)</td>
<td>5.80 ± 0.79(^c)</td>
<td>0.996</td>
</tr>
</tbody>
</table>

\(^1\)Values represent the mean ± standard deviation of triplicate tests.

\(^2\)Values in the same column for the parameters followed by different superscripts are significantly different (\(p < 0.05\)). It should be noted that the response variables (\(k\), \(\beta\), \(t_{1/2}\) and \(t_{lag}\)) were statistically influenced only by the diet variety (at the same mucin concentration) and the mucin concentration (within the same diet), so different diets with addition of different mucin concentrations should not be compared directly.
Figure captions

**Fig. 1.** A: Installation diagram of the dynamic in vitro rat stomach-duodenum (DIVRSD) model. (1) soft-elastic silicon rat stomach model; (2) angled plate; (3) eccentric wheel; (4) driving shaft; (5) stepper motor; (6) bevel gear; (7) belt; (8) silicon rat duodenum model; (9) pulley system; (10) frequency controller; (11) tube for collection of duodenal digesta; (12) syringe pump; (13) temperature-controlled box; B: Schematic diagram showing the details of the DIVRSD model.

**Fig. 2.** A: Changes in pH of the gastric and duodenal digesta of the control, pectin and mango diets with various dry matter contents (%) during digestion in the DIVRSD model; B: Gastric emptying (as expressed by fractional retention in stomach) of the control, pectin and mango diets with various dry matter contents in the DIVRSD model (The emptying data is fitted with the modified Elashoff’s model).

**Fig. 3A.** Storage modulus (G’) and loss modulus (G”) of the gastric and duodenal digesta of the control, pectin and mango diets with various dry matter contents (%) after in vitro digestion in the DIVRSD model for 30, 60 and 120 min.

**Fig. 3B.** Dynamic and steady shear viscosity of the gastric and duodenal digesta of the control, pectin and mango diets with the same dry matter contents (%) after in vitro digestion in the DIVRSD model for 30, 60 and 120 min.

**Fig. 4.** Starch and protein hydrolysis (%) of the control, pectin and mango diets with various dry matter contents (%) during digestion in the DIVRSD model. Hydrolysis is measured for duodenal digesta and represents the combined effect of digestion in both gastric and duodenal phases.

**Fig. 5.** SEM images of the duodenal digesta of the control (A), pectin (B) and mango (C) diets obtained from 0, 30 and 120 min digestion in the DIVRSD model. The red arrows shown in the control and mango diets indicate the small pores on the surface of starch granules.

**Fig. 6.** Rheology of the duodenal digesta of the control, pectin and mango diets with the same dry matter content of 20.35% in the presence of 0, 10 and 20 mg/mL porcine gastric mucin after 30 min digestion in the DIVRSD model. $\eta^*$-dynamic viscosity, $\eta_{\text{app}}$-apparent viscosity or steady shear viscosity.

**Fig. 7.** Gastric emptying as well as starch and protein hydrolysis of the pectin diet with a dry matter content of 20.35% in the presence of of 0, 10 and 20 mg/mL porcine gastric mucin.
Highlights

- An improved dynamic rat stomach-duodenum model is developed
- Low digestion and gastric emptying rates in pectin-enriched diet
- High modulus and viscosity of digesta from pectin-enriched diet
- Starch granules in pectin diet trapped in gel network during digestion
- Gastric mucin reduced starch/protein hydrolysis and gastric emptying rates