Carbohydrate Polymers 84 (2011) 162–172

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Weighing up whey fortification of foods: Implications for kinetics of starch digestion and estimated glycemic index of model high-protein-low-carbohydrate food systems☆

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ARTICLE INFO

Article history:
Received 27 September 2010
Received in revised form 4 November 2010
Accepted 9 November 2010
Available online 18 November 2010

Keywords:
In vitro starch digestion
First-order kinetic model
Whey protein isolate
Wheat starch
Digestion model
Glycemic load

ABSTRACT

Mixtures of whey protein isolate (20 and 50%) and wheat starch, as a model high-protein-low-carbohydrate (HPLC) food, were extruded in a twin-screw extruder by changing moisture, temperature and screw speed. Longitudinal expansion increased with whey, and screw speed enhanced transverse expansion. *In vitro* starch digestion revealed monophasic digestograms for extrudates and non-extrudates. A modified first-order kinetic model suitably predicted the starch digestograms. Whey concentration of the non-extrudates did not significantly affect the digestion parameters, but extrusion increased these parameters. In the extrudates, whey increased (very) rapidly digested starch and glycemic index (GI), while rate of digestion and glycemic load (GL) reduced. Whey-fortification can increase bioactives in HPLC foods, but there is a need to increase resistant starch in such foods for maximum benefits. Being possibly the first study on the kinetics of starch digestion in whey–starch formulations, the present study highlights ways of increasing resistant starch in HPLC foods.

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1. Introduction

Snacks or ready-to-eat (RTE) foods are mainly cereal-based and widely available, making them an important part of the global diet. However, their high digestible starch content raises nutritional and health issues (Cho & Rizvi, 2010), leading to suggestions to fortify high-starch RTE food with proteins. Various studies have been conducted with quality proteins to produce high-protein-low-carbohydrate (HPLC) foods for the promotion and maintenance of good health (Ainsworth, Ibanoglu, Plunkett, Ibanoglu, & Stojceska, 2007; Amaya-Llano, Hernandez, Tostado, & Martinez-Bustos, 2007; Cho & Rizvi, 2010). Although plant and animal proteins were investigated, the functional properties of whey proteins, and the need to increase their global utilisation, make them more suitable to fortify starch products (Hoppe et al., 2008; McIntosh et al., 1998; Onwulata, Smith, Konstance, & Holsinger, 2001). Functional properties claimed for whey proteins include anti-oxidant, immunostimulatory, anti-carcinogenic, hypocholesterolaemic, inhibition of angiotensin-converting-enzyme (ACE), gut cell protection, and digestive functions (Hoppe et al., 2008; McIntosh et al., 1998; Vermeirssen, Van Camp, & Verstraete, 2002).

Snack foods are mainly processed by extrusion, and whey–starch/cereal extrudates have featured prominently in the literature (Amaya-Llano et al., 2007; Chaiyakul, Jangchud, Jangchud, Wuttijumnong, & Winger, 2009; Kim & Maga, 1987; Onwulata, Konstance, Smith, & Holsinger, 2001; Onwulata, Smith, et al., 2001). However, these studies concentrated on how whey proteins influenced physicochemical (e.g. expansion and composition), textural (e.g. breaking strength and cohesiveness) and functional (e.g. water absorption and solubility indices) characteristics of extrudates. The effects of whey fortification on extruder response (e.g. pressure and specific mechanical energy) have also been reported. During heat–moisture–shear–(pressure) treatments as obtained in extrusion, starch and protein can respectively be gelatinised and denatured, with the possibility of starch–protein complexes (Allen, Carpenter, & Walsh, 2007; Matthey & Hanna, 1997; Onwulata, Smith, et al., 2001; Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998; Shim & Mulvaney, 2001; Zhang, Maladen, & Hamaker, 2003). These transformations influence digestibility of starch, protein and their mixtures, and although *in vitro* protein digestibility of whey fortified foods has been reported (e.g. Amaya-Llano et al., 2007), starch digestibility in such foods

☆ Part of this paper was presented at the 41st Annual Conference of the Australian Institute of Food Science and Technology, Sydney, Australia 21–24 July 2008.

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doi:10.1016/j.carbpol.2010.11.020

Nomenclature

D_0	Digested starch at time $t = 0$; very rapidly digested starch, VRDS (g per 100 g dry starch)
D_t	Digested starch at time t (g per 100 g dry starch)
D_∞	Digested starch at infinite time $t = \infty$; maximum digested starch (g per 100 g dry starch)
d_d	Extruder die diameter (m)
G	Mass flow rate (kg s^{-1})
GI	Glycemic index (%)
GL	Glycemic load (g per g solids)
K	Rate constant (min^{-1})
P_R	Power rating of the extruder motor (W)
SS	Screw speed (rpm)
S	Starch content of sample (g per 100 g solids)
SS_{MAX}	Maximum extruder screw speed (rpm)
t	Time (min)
Γ	Torque (%)

has not received comparable attention. It is well recognised that although single-point starch digestion is valuable, time-course (or kinetics) measurements reveal more information on, for example, the rate and extent of digestion (Mahasukhonthachat, Sopade, & Gidley, 2010a), from which glycemic index can be estimated (Goñi, Garcia-Alonso, & Saura-Calixto, 1997). We are not aware of any detailed studies on how whey proteins affect glycemic index and load of RTE food. Therefore, the objectives of this study were to:

i. Investigate starch digestion in extruded and non-extruded mixtures of whey protein isolate and wheat starch.
ii. Model the relationship between digested starch and time of digestion in a simulated gastro-intestinal tract.
iii. Examine how starch digestion parameters explain the effects of extrusion on starch digestibility in model high-protein-low-carbohydrate foods.

2. Materials and methods

2.1. Materials

Whey protein isolate (W), ALACAN™ 894, was obtained from Total Foodtec Marketing Pty. Ltd., Darra QLD 4076, Australia and contains (manufacturer's data) 90.5% protein, 0.2% fat, 1.1% total carbohydrate, 3.2% ash, and pH of 6.80. Wheat starch (S), Wheaten cornflour™, was purchased from Penford Australia Ltd., Lane Cove, NSW 2066, Australia. Prior to processing, 20% whey protein isolate–80% wheat starch (20W80S) and 50% whey protein isolate–50% wheat starch (50W50S) mixtures were thoroughly mixed using gently rotating rollers (5–7 days; 4 rpm), before their moisture (vacuum oven method) and protein (LECO nitrogen combustion method) contents were determined (%) as: 20W80S-moisture, 10.4; protein, 20.9; 50W50S-moisture, 9.0; protein, 48.0. The moisture and protein contents (%) of the wheat starch (100S) were respectively measured to be 11.4 and 0.5.

2.2. Extrusion

The whey–starch mixtures (20W80S and 50W50S) were extruded in a Prism Eurolab KX16 twin screw extruder (Thermo Prism, Emerald Way ST15 0SR, UK) using the screw configuration and temperature profile in Table 1. The descriptions of the extruder and how it was run are available elsewhere (Mahasukhonthachat et al., 2010a). We concentrated on whey–starch mixtures because starch extrusion is a relatively well-researched area, and our interests were to understand the digestibility of whey–starch extrudates. The total feed rate (powder and water) was fixed at 1.2 kg/h, while moisture (30 and 50%), screw speed (150, 220 and 300 rpm) and maximum barrel temperatures (110, 140 and 160 °C) were varied in a randomised full factorial nested experimental design ($2 \times 2 \times 3 \times 3 = 36$) for the two whey–starch (20W80S and 50W50S) formulations. The formulation was nested within temperatures, and moisture and screw speed were nested within formulation. The full factorial experiment was not replicated, but the experimental error was estimated from three replicates of the control conditions of wheat starch (100S) at 50% moisture, 300 rpm and maximum barrel temperature of 110 °C. The analysis was done with the design-of-experiment (DOE) option in Minitab®.

Extrudates were collected after equilibration of extrusion (stable torque and pressure) and cooled overnight at room temperature. The cooled extrudates were immediately analysed for their physical properties (transverse and longitudinal expansion) as described below prior to storage at −20 °C in polythene bags. A quartering procedure was used to obtain a sample of each extrudate, and each sample was freeze-dried and cryomilled as

Table 1
Screw configuration and temperature profile of the extruder.

Inlet	Temperature (°C)	L:D	Screw element
Feed	RT	1	Feed screw
		2	Feed screw
		3	Feed screw
		4	Feed screw
	50	5	Feed screw
		6	Feed screw
		7	Feed screw
		8	Feed screw
	50	9	Feed screw
Water		10	Feed screw
		11	Feed screw
		12	Feed screw
	75	13	Feed screw
		14	4 x 60° Forward paddles
		15	Feed screw
		16	Feed screw
	90	17	Feed screw
		18	Feed screw
		19	4 x 60° Forward paddles
		20	4 x 60° Forward paddles
	110, 140 or 160	21	4 x 90° Forward paddles
		22	Feed screw
		23	Feed screw
		24	Feed screw
	110, 140 or 160	25	Feed screw
		26	Feed screw
		27	Feed screw
		28	4 x 60° Forward paddles
		29	4 x 60° Forward paddles
	110, 140 or 160	30	4 x 90° Forward paddles
		30.5	0.5 Feed screw
		31.5	Feed screw
		32.5	Feed screw
	105	33.5	Feed screw
		34.5	Feed screw
		35.5	Feed screw
	100	36.5	Feed screw
		37.5	Feed screw
		38.5	Feed screw
		40	1.5 Lock Forward screw
	Unheated		2 x 2 mm Die block

[a] RT = zone 1 of the barrel was maintained at room temperature by circulating domestic pipe borne (tap) water.

described before (Mahasukhonthachat et al., 2010a), prior to analysis.

2.3. Extruder response and physical properties of extrudates

The specific mechanical energy (SME) was defined as:

$$\mathrm{SME} = \left(\frac{\mathrm{SS}}{\mathrm{SS_{MAX}}}\right) \times \left(\frac{\Gamma}{100}\right) \times \left(\frac{P_R}{G}\right) \quad (1)$$

The no-load torque was negligible, and SME was calculated from the data logged every 15 s for not less than 10 min per extrusion condition.

Transverse expansion (TE, $\mathrm{m\,m^{-1}}$) was defined as the diameter of the extrudate relative to the die diameter (d_d), while longitudinal expansion (LE, $\mathrm{m\,kg^{-1}}$) was defined as length per unit weight of extrudate, and apparent density (TD, $\mathrm{kg\,m^{-3}}$) was calculated as in Eq. (2):

$$\mathrm{TD} = \frac{4}{(\pi \times \mathrm{LE} \times d_d^2 \times \mathrm{TE}^2)} \quad (2)$$

2.4. In vitro starch digestion

Starch digestion was assayed using the rapid *in vitro* starch digestion procedure of Sopade and co-workers (Mahasukhonthachat et al., 2010a; Mahasukhonthachat, Sopade, & Gidley, 2010b; Sopade & Gidley, 2009). The procedure briefly involves treating about 500 mg of milled or powder sample with artificial saliva (porcine α-amylase in carbonate buffer, pH 7) and pepsin at pH 2 prior to incubation at 37 °C for 30 min (salivary-gastric digestion) in a reciprocating (85 rpm) water bath. The digesta was neutralised with NaOH before adjusting the pH to 6.0 with sodium acetate buffer before a mixture of pancreatin and amyloglucosidase enzymes was added, and incubated for up to 240 min (small intestine or pancreatic digestion). The glucose released as a result of starch digestion was measured with an Accu-Check® Performa® glucometer (Roche Diagnostics Australia Pty. Ltd., Caste Hill NSW 2154, Australia), and digested starch (g per 100 g dry starch) at a measurement time (min) was calculated as before (Sopade & Gidley, 2009).

2.5. Total starch content

The total starch content of the samples was analysed using a method derived from Megazyme (Megazyme International Ireland Ltd., Wicklow, Ireland) as described before (Mahasukhonthachat et al., 2010b; Sopade & Gidley, 2009). About 50 mg of the sample was wetted with ethanol before it was heated in a boiling water bath in the presence of dimethyl sulphoxide. The solubilised and gelatinised starch was digested with thermostable α-amylase in MOPS before sodium acetate buffer and amyloglucosidase were added prior to incubation (50 °C). The glucose, and hence, starch content, were determined using an enzymatic glucose reagent and measuring absorbance at 505 nm against a reagent blank.

2.6. Scanning electron microscopy

Scanning electron microscopy (environmental, E-SEM) of the extrudates was conducted in a TM-1000 Tabletop SEM (Hitachi High-Technol. Corp., Tokyo) at 15 kV accelerating voltage and 28.6 mA emission current. This is an environmental SEM (E-SEM), and the extrudates were viewed without coating at a low vacuum.

2.7. Modelling starch digestograms

A modified first-order kinetic model, Eq. (3), was used to describe the starch digestograms (Mahasukhonthachat et al., 2010b):

$$D_t = D_0 + D_{\infty-0}(1 - \exp[-Kt]) \quad (3)$$

The Microsoft Excel Solver® was used to compute the parameters of the model by minimising the sum of squares of residuals (SUMSQ) and constraining $D_\infty \leq 100$ g/100 g dry starch, and $D_0 \geq 0$ g/100 g dry starch. In addition to the coefficient of determination (r^2), the predictive ability of the models was assessed with the mean relative deviation modulus (MRDM) as described before (Mahasukhonthachat et al., 2010b). In order to calculate glycemic indices of the samples, the areas under the digestograms ($\mathrm{AUC_{exp}}$) were computed ($t_1 = 0$, $t_2 = 240$ min) with Eq. (4), which is the integral form of Eq. (3):

$$\mathrm{AUC_{exp}} = \left[D_\infty t + \frac{D_{\infty-0}}{K}\exp(-Kt)\right]_{t_1}^{t_2} \quad (4)$$

The hydrolysis index (HI) of each sample was calculated by dividing the area under its digestogram by the area under the digestogram of a fresh white bread (Goñi et al., 1997). In an unpublished study in our laboratories using the procedures in 2.4, we obtained the area under the digestogram of fresh white bread to be ≈13 000 min g/100 g dry starch from 0 to 240 min. From Goñi et al. (1997), single-point measurements of starch digestion at 90 min in samples can also be used to estimate GI ($\mathrm{GI_{H_{90}}}$). Hence, using the parameters of the modified first-order kinetic model (Eq. (3)) for both the samples and fresh white bread, GIs ($\mathrm{GI_{H_{90}}}$ and $\mathrm{GI_{HI}}$) of the samples were also calculated, and the average GI (AVG-GI) for each sample was defined as below (Eq. (5)):

$$\mathrm{GI} = \frac{[(39.21 + 0.803\,\mathrm{H_{90}}) + (39.51 + 0.573\,\mathrm{HI})]}{2} \quad (5)$$

The glycemic load per g solids (GL) was calculated from the average GI as in Eq. (6):

$$\mathrm{GL} = \mathrm{GI} \times \frac{\mathrm{S}}{100} \quad (6)$$

2.8. Statistical analysis

Analysis of variance (ANOVA) and test of significance were performed using Minitab® ver. 16 with the overall confidence level of 95%. In the ANOVA, the four-order interactions of the extrusion conditions (formulation × moisture × temperature × speed) were used as an error term. Except otherwise stated, samples were randomised and duplicated for all the analyses. For extrudate expansion (2.3), not less than 10 strands per extrudate were used.

Statistical analysis of the starch digestograms was conducted in three stages:

(a) Salivary-gastric and pancreactic digestion (subscript $_{\mathrm{ALL}}$) were analysed together, when the experimental time-course digested starch data were used as obtained.
(b) Pancreactic digestion (subscript $_{\mathrm{PANC}}$), when the experimental digested starch at time $t = 0$ (salivary-gastric digestion) was subtracted from the experimental time-course digested starch.
(c) Pancreactic digestion relative to the non-extrudates (subscript $_{\mathrm{PANC,RAW}}$), when the predicted (from Eq. (3)) digested starch at time $t = 0$ (salivary-gastric digestion) for the non-extruded samples was subtracted from the experimental time-course digested starch of the extrudates.

The digestograms were analysed in stages (b) and (c) to nullify the effects, if any, of existing glucose in the samples or from

the non-extrudates. Moreover, although according to the manufacturer, the whey protein isolate was low in carbohydrate, possibly lactose, Sopade and Gidley (2009) observed that lactose could affect the readings of the glucometer used in the *in vitro* starch digestion. These authors opined that if low molecular weight carbohydrates are suspected to affect the readings from the glucometer, digested starch data related to pancreatic digestion should be used to model the digestograms (Sopade & Gidley, 2009).

3. Results and discussion

3.1. Reproducibility of the extrusion process

From the three replicates of 100S at 50% moisture, 300 rpm and maximum barrel temperature of 110 °C, the extrusion process had a coefficient of variation from time-course digested starch, specific mechanical energy (SME) and extrudate expansion of less than 16% (e.g. longitudinal expansion = $140 \pm 20\,m\,kg^{-1}$; transverse expansion = $1.5 \pm 0.2\,m\,m^{-1}$; apparent density = $1080 \pm 170\,kg\,m^{-3}$; SME = $290 \pm 30\,kJ\,kg^{-1}$). For these replicates, the die pressures were 1–2 bar (0.1–0.2 $MN\,m^{-2}$). In the absence of extrudate collapse, high die pressures could yield high transverse expansion (Amaya-Llano et al., 2007; Onwulata, Smith, et al., 2001; Sopade & Le Grys, 1991). The low die pressure with these replicates was possibly because of high moisture and low maximum barrel temperature, despite possibly high frictional heat from the high screw speed.

3.2. Extruder response and extrudate expansion

SME is the sum of the energy in shearing and pumping extruder feed, and the energy in turning the empty extruder (Liang, Huff, & Hsieh, 2002). The dependence of SME on process conditions during whey–starch extrusion has been investigated, and possibly because of differences in screw configuration, extrusion conditions and feed properties, there were decreases and increases in SME with whey protein (Matthey & Hanna, 1997; Onwulata, Smith, et al., 2001; Onwulata, Konstance et al., 2001). In the present study, SME was not significantly ($p > 0.05$) affected by the concentration of whey in the mixtures (Table 2). However, at 50% moisture, 300 rpm screw speed and 110 °C maximum barrel temperature, SME of the whey–starch mixtures ($\cong 130\,kJ\,kg^{-1}$) was much less than that for the wheat starch ($290\,kJ\,kg^{-1}$). This agrees with the observations of Sopade, Hardin, Fitzpatrick, Desmee, and Halley (2006) on the reduction of RVA pasting viscosity of the wheat starch as the concentration of whey proteins was increased. However, it is recognised that melt viscosity (limited water) in extrusion is different from RVA pasting viscosity (excess water). In accordance with its effects on melt viscosity, moisture content reduced SME, which increased with an increase in screw speed (Fig. 1a). SME could increase with speed because more mechanical energy would be required to turn the screws, particularly when the reduction in torque due to frictional heat and reduced melt viscosity, is incommensurate with the mechanical energy demand (Mahasukhonthachat et al., 2010a).

The main effects of formulation, temperature, moisture, and screw speed significantly ($p \leq 0.05$) affected expansion (Table 2). LE increased with whey concentration (Fig. 1b), which reduced TE. However, extruding at 50% moisture, 300 rpm and maximum barrel temperature of 110 °C, TE of 100S and 50W50S were not significantly different ($p > 0.05$), but TE of 100S was less than 20W80S. This possibly indicates that above a critical level, proteins are detrimental to TE due to physical, structural, chemical, and molecular changes (Amaya-Llano et al., 2007; Chaiyakul et al., 2009). Screw speed enhanced TE (Fig. 1c), while temperature was detrimental to TD (Fig. 1d). Although an increase in barrel temperature increased TE, LE increased with temperature up to 140 °C before decreasing to 160 °C (not shown). The combined effects of temperature on LE and TE could have resulted in its detrimental effects on TD. Previous studies have shown that a critical temperature appears to exist, above which expansion reduces (Amaya-Llano et al., 2007) because of temperature-induced physicochemical transformations in starch–protein systems. Although TE is more commonly used, both TE and LE reveal different properties of the melt that control expansion, and both are important in correct assessments of extrudate expansion. It can be observed (Fig. 1c insert; $LE = 270\,(TE)^{-1.5}$; $r^2 = 0.815$; $p < 0.001$) that for the whey–starch extrudates, both LE and TE were inversely related irrespective of the whey concentration. Hence, as discussed in various studies (e.g. Launay & Lisch, 1983; Mahasukhonthachat et al., 2010a), longitudinal and transverse expansion, and consequently extrudate density or specific volume, which are quality and acceptability parameters, are differently affected by viscoelastic properties of the melt.

Generally, quality and acceptability of whey–starch extrudates are also dependent on other physical (and functional) properties, such as water absorption and solubility indices, pasting properties and gelatinisation characteristics. We observed that these have been well researched by previous authors (e.g. Amaya-Llano et al., 2007; Kim & Maga, 1987; Matthey & Hanna, 1997; Onwulata, Konstance et al., 2001; Onwulata, Smith, et al., 2001), and are not discussed in the present study, whose focus is the kinetics of starch digestion in the whey–starch systems.

3.3. In vitro starch digestibility

Irrespective of the concentrations of whey and extrusion conditions, whey–starch mixtures exhibited monophasic digestograms (Fig. 2). Generally and consistent with theory, the extrudates were better digested than the non-extrudates, because extrusion, like all heat-moisture treatments, gelatinises starch. In the presence of favourable factors, gelatinisation improves starch digestibility (Chung, Lim, & Lim, 2006; Faraj, Vasanthan, & Hoover, 2004; González-Soto, Mora-Escobedo, Hernández-Sánchez, Sánchez-Rivera, & Bello-Pérez, 2007; Holm, Lundquist, Björck, Eliasson, & Asp, 1988; Sun, Lærke, Jørgensen, & Knudsen, 2006). The dependence of the rate and extent of starch digestibility on feed properties and extrusion conditions are further clarified by modelling the starch digestograms to obtain the digestion parameters, and highlight specific effects.

3.4. Modelling starch digestograms

The major digestion models have been reviewed by Mahasukhonthachat et al. (2010b), who also discussed the limitations of the Michaelis–Menten model in describing multi-enzyme *in vitro* and *in vivo* digestograms. These limitations make semi-theoretical and empirical models the only suitable ones for modelling digestograms. Monophasic digestograms (Fig. 2) are more commonly exhibited by materials, but Liu, Sabboh, Kirchhoff, and Sopade (2010) reported biphasic digestograms in sweet potato, which have recently been modelled using a logistic model or modelled as two monophasic digestograms using the first-order kinetic model (Liu & Sopade, unpublished results). The first-order kinetic model is widely used, and this can be modified (Eq. (3)) to incorporate salivary-gastric digestion and estimate very rapidly digested starch (VRDS). Various studies in our laboratories have shown that salivary-gastric digested starch or VRDS is not always negligible, particularly when *in vitro* procedures has a salivary-gastric step, just as the maximum digested starch is not

Table 2
Effect of extrusion on extruder response, expansion property and kinetics of starch digestion.[a]

Main and interactions factors	SME	LE	TE	TD	D_0	D_∞	K	$GI_{H_{90}}$	GI_{HI}	AVG-GI	GL
Formulation	NS	S**	S*	NS	S***	S***	S***	S***	S***	S***	S***
Temperature	NS	S*	S**	S*	NS	NS	NS	NS	NS	NS	NS
Moisture	S***	S**	NS	NS	S*	S*	NS	NS	NS	NS	NS
Screw speed	S*	NS	S*	NS	NS	S*	NS	NS	NS	NS	NS
Formulation × temperature	S*	NS	NS	S*	NS	NS	NS	NS	NS	NS	NS
Formulation × moisture	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Formulation × screw speed	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Temperature × moisture	NS	NS	S*	NS	NS	NS	NS	NS	NS	NS	NS
Temperature × screw speed	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Moisture × screw speed	S*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Formulation × temperature × moisture	NS	NS	NS	S*	NS	NS	NS	NS	NS	NS	NS
Formulation × temperature × screw speed	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Temperature × moisture × screw speed	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a] SME = specific mechanical energy (kJ kg^{-1}), TE = transverse expansion (m m^{-1}), LE = longitudinal expansion (m kg^{-1}), TD = apparent density (kg m^{-3}), D_0 = very rapidly digested starch ($t = 0$) (g/100 g dry starch), D_∞ = maximum digested starch ($t \to \infty$) (g/100 g dry starch), K = rate of starch digestion (min^{-1}), $GI_{H_{90}}$ = glycemic index using digested starch at 90 min, GI_{HI} = glycemic index using hydrolysis index relative to white bread, AVG-GI = average glycemic index (Eq. (5)), GL = glycemic load, NS = non-significant. These apply to all tables and figures where they appear.
* Significant at $p \le 0.05$.
** Significant at $p \le 0.01$.
*** Significant at $p \le 0.001$.

always 100% as demonstrated in other studies (e.g. Goñi et al., 1997).

The modified first-order kinetic model (Eq. (3)) suitably predicted the digestograms of both the extrudates and non-extrudates (Fig. 2), with $r^2 = 0.946$, MRDM = 9, SUMSQ = 272. Both the Peleg and Duggleby models, described elsewhere (Mahasukhonthachat et al., 2010b) were also found to be suitable in describing the digestograms, but the present discussion will concentrate on the modified first-order model. Table 3 shows the specific values of the parameters of the model (Eq. (3)) as they varied with extrusion. The parameters of the model were generally higher for the extrudates than non-extrudates. This is expected, and as explained above, from the effects (gelatinisation and dextrinisation) of extrusion on starch. Fig. 3 confirms the loss of the native starch and protein structures in the extrudates, thereby enhancing starch digestion. Within the limits of experimental errors, even though the extrudates would be digested faster (higher K, min^{-1}), both the extrudates and non-extrudates would be digested to effectively the same maximum extent (D_∞) of 100 g per 100 g dry starch or less. Englyst, Kingman, and Cummings (1992) classified raw

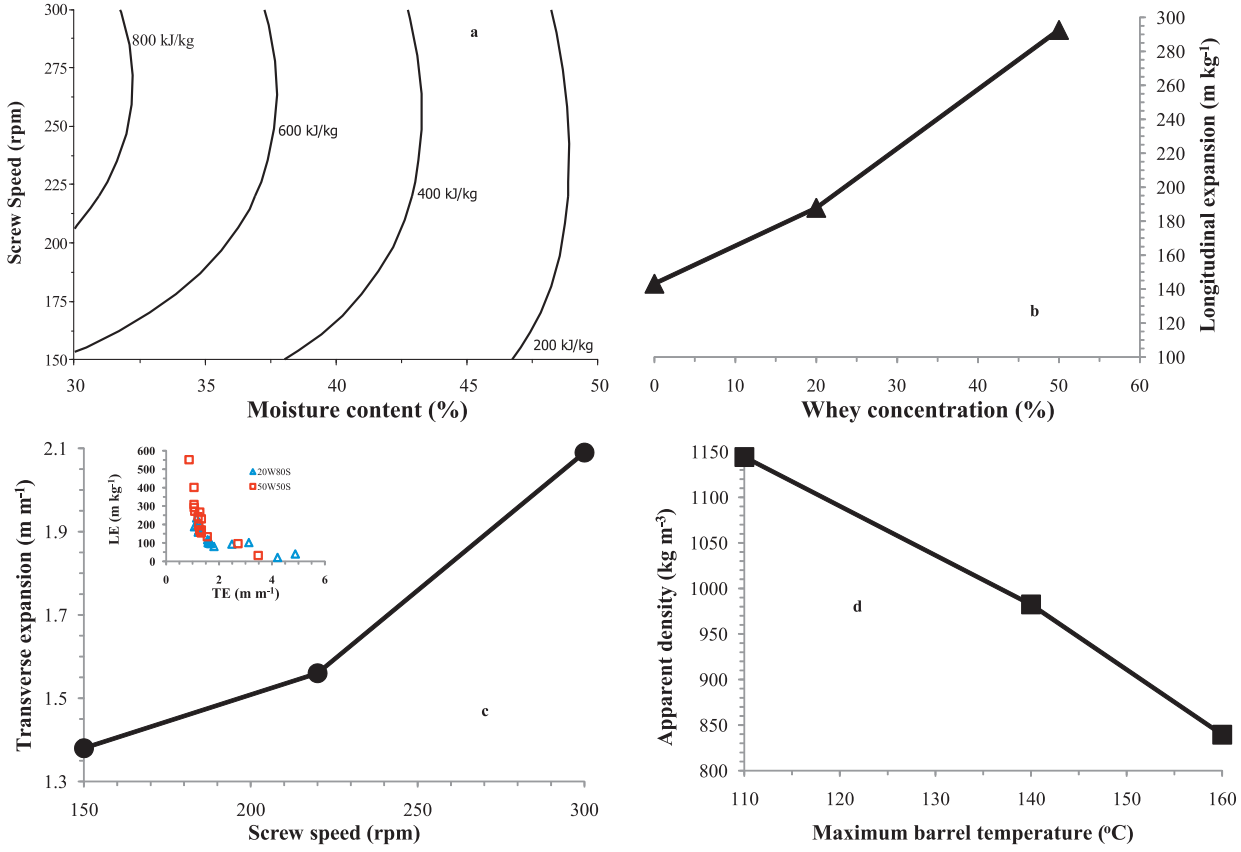

Fig. 1. Relationships between extruder response, expansion properties and extrusion conditions. (a) Specific mechanical energy, (b) Longitudinal expansion, (c) Transverse expansion, (d) Apparent density.

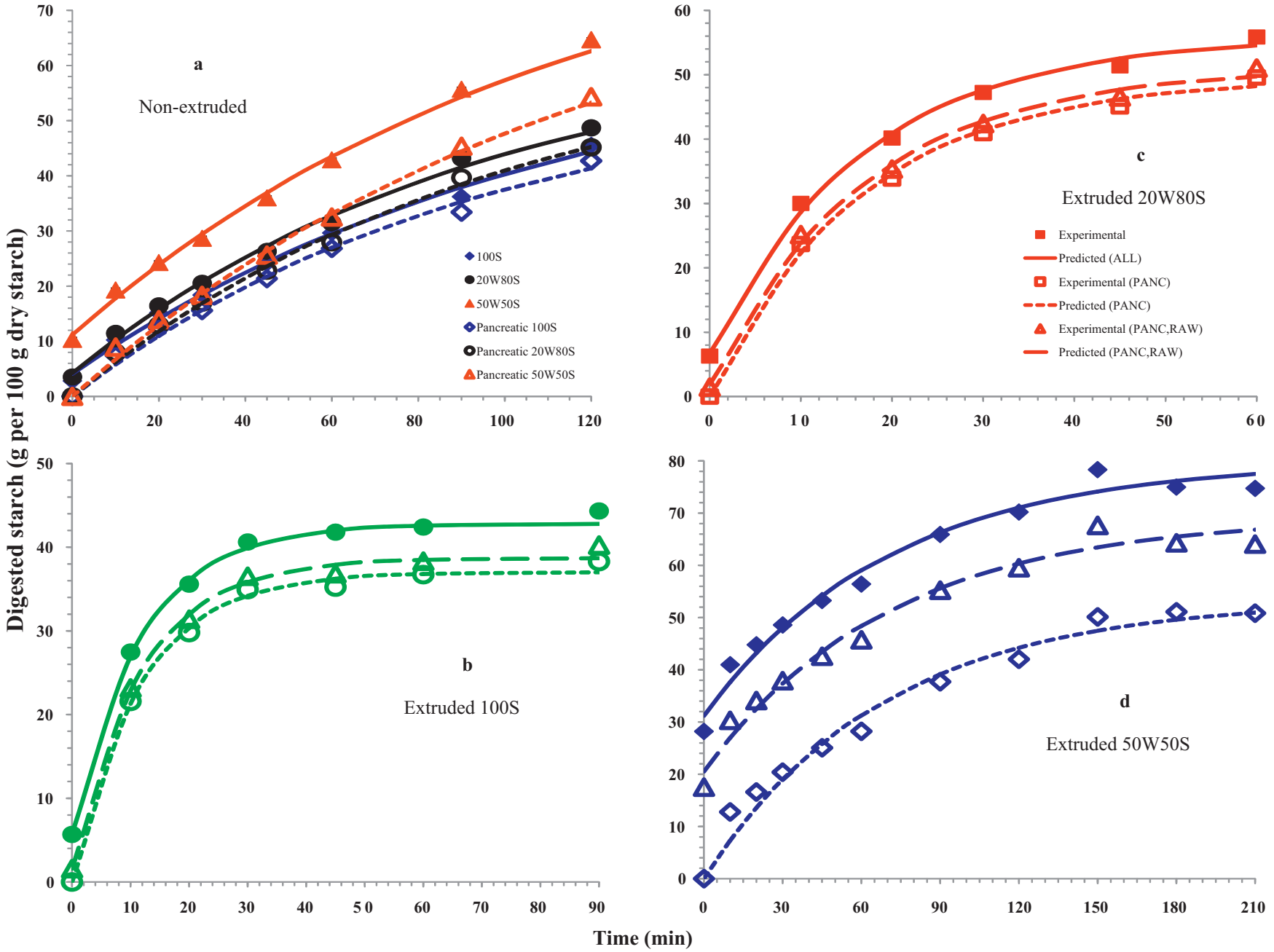

Fig. 2. Digestograms of the extruded and non-extruded whey–starch samples showing the predictions from the modified first-order kinetic model. (a) Non-extrudate (S = starch, P = protein), (b) Extruded 100S at 50% moisture, 300 rpm and 110 °C maximum barrel temperature, (c) Extruded 20W80S at 50% moisture, 300 rpm and 110 °C maximum barrel temperature, (d) Extruded 50W50S at 50% moisture, 300 rpm and 110 °C maximum barrel temperature. The error bars are not shown for clarity, but coefficient of variation, CV (100 × standard deviation/mean) averaged less than 10%.

Table 3
Parameters of the digestion models for the extrudates.[a]

Extrusion parameter		D_0	D_∞	$K \times 10^{-3}$	$GI_{H_{90}}$	GI_{HI}	AVG-GI	GL
Salivary-gastric and pancreatic digestion ($_{ALL}$)								
Whey concentration (%)	20	8.4 b	53.1 b	66.7 a	82 b	91 a	86 b	69 a
	50	31.8 a	83.8 a	15.9 b	96 a	112 b	104 a	52 b
Temperature (°C)	110	19.5 a	68.8 a	40.1 a	89 a	102 a	96 a	61 a
	140	20.9 a	69.0 a	42.4 a	89 a	102 a	96 a	61 a
	160	19.7 a	67.5 a	41.3 a	88 a	101 a	95 a	60 a
Moisture content (%)	30	20.6 a	69.5 a	41.5 a	89 a	102 a	96 a	61 a
	50	19.5 b	67.4 b	41.0 a	88 a	101 a	95 a	60 a
Screw speed (rpm)	150	20.0 a	68.5 a b	38.6 a	90 a	102 a	96 a	61 a
	220	20.2 a	70.1 a	40.5 a	89 a	102 a	96 a	61 a
	300	20.1 a	66.8 b	44.7 a	88 a	100 a	94 a	60 a
Pancreatic (subtracting $D_{0,PANC}$)								
Whey concentration (%)	20	0	45.8 b	67.0 a	76 a	84 a	80 a	64 a
	50	0	54.5 a	18.3 b	74 a	83 a	79 a	39 b
Temperature (°C)	110	0	51.2 a	41.6 a	76 a	84 a	80 a	52 a
	140	0	50.0 a	43.0 a	75 a	83 a	79 a	51 a
	160	0	49.3 a	43.3 a	75 a	83 a	79 a	51 a
Moisture content (%)	30	0	50.4 a	43.6 a	75 a	83 a	79 a	52 a
	50	0	50.0 a	41.7 a	75 a	84 a	79 a	52 a
Screw speed (rpm)	150	0	49.7 a b	43.1 a	75 a	84 a	80 a	52 a
	220	0	51.8 a	40.8 a	76 a	84 a	80 a	52 a
	300	0	49.0 b	44.0 a	74 a	82 a	78 a	51 a
Subtracting D_0 of the non-extrudate ($_{PANC,RAW}$)								
Whey concentration (%)	20	3.7 b	48.1 b	68.1 a	78 b	86 b	82 b	66 a
	50	21.1 a	73.2 a	15.9 b	88 a	101 a	94 a	47 b
Temperature (°C)	110	11.9 a	60.8 a	42.4 a	83 a	94 a	88 a	56 a
	140	13.2 a	61.3 a	42.4 a	83 a	94 a	89 a	56 a
	160	12.1 a	59.8 a	41.1 a	82 a	93 a	88 a	56 a
Moisture content (%)	30	12.9 a	61.6 a	43.1 a	83 a	94 a	89 a	57 a
	50	11.8 a	59.7 a	40.9 a	82 a	93 a	88 a	56 a
Screw speed (rpm)	150	12.4 a	60.5 a b	40.8 a	83 a	94 a	89 a	57 a
	220	12.4 a	62.3 a	40.5 a	83 a	94 a	89 a	57 a
	300	12.3 a	59.0 b	44.7 a	82 a	92 a	87 a	56 a

[a] For each extrusion main effect, figures with the same letters are not significantly different ($p > 0.05$).

starch as resistant starch type 1, and (processed but) retrograded starch as type 3. Extrusion is widely reported (e.g. Knudsen, Lærke, Steenfeldt, Hedemann, & Jørgensen, 2006) to yield retrograded starch.

Starch retrogradation is generally enhanced by high-moisture processing as amylose rapidly re-associates in the presence of more binding sites. However, in dilute gelatinised starch systems, the molecules might be far apart to prevent re-association that limits retrogradation. When it occurs, retrogradation limits starch digestion, with a concomitant reduction in glycemic index, and for the same starch content, glycemic load per unit dry weight. However, it can be observed in Table 3 that, apart from D_0 and D_∞, the digestion parameters were not dependent on extrusion moisture, temperature and screw speed. Even though the rate of digestion was not significantly different, the observations with D_0 and D_∞ suggested that the extrudates were more digestible at 30% moisture content than at 50%. This is consistent with the effects of retrogradation, which was possibly enhanced at the higher moisture level. Moreover, the inverse relationship between extrusion moisture and digestion can also be explained (Mahasukhonthachat et al., 2010a), from its reducing effects on SME (Fig. 1a), which measures the overall shear input.

For both extrudates and non-extrudates, the amount of whey in the feed exercised the most significant effect on all the digestion parameters. Generally, the higher the whey concentration, the better was starch digestion (Table 3). It can be observed that the effects of whey concentration were more with the extrudates possibly because extrusion or processing enhanced whey–starch interactions as against physical mixing of starch and proteins in the non-extrudates. This could be partly because, as discussed above in Fig. 3, both protein and starch native structures, which were obvious in the non-extrudates, were indistinguishable in the extrudates. Possibly because of structural and/or molecular reasons, exogenous proteins, and specifically whey proteins, aid digestion (Amaya-Llano et al., 2007; Basha & Palanivelu, 1998; Sharma & Khetarpaul, 1995). However, these authors did not model the resulting digestograms to gauge specific effects of substituting carbohydrates with whey proteins for the production of high-protein-low-carbohydrate foods. This is important for the overall benefits of whey proteins and resistant starch.

3.5. General discussion

Whey proteins are rich in bioactives that are important in nutrition and health. Nutritional and health benefits of resistant starch have been widely proven. Hence, in HPLC foods, high resistant starch and bioactives are desirable. It is, therefore, important, to examine the roles of whey concentration on starch digestion in the present study. Fig. 4 shows the trend with the non-extrudates from 0 to 50% whey fortifications, while Fig. 5 shows how the digestion parameters varied when the three fortifications when extruded at 50% moisture, 300 rpm and maximum barrel temperature of 110 °C. For both extrudates and non-extrudates, even if nominally, whey increased very rapidly digested starch (D_0), maximum digested starch (D_∞), and glycemic index (GI), but reduced the rate of digestion (K) and glycemic load (GL). Although the reduction in GL and K are beneficial to the nutrition and health properties of HPLC foods, the increase in D_0, D_∞ and GI, appear to negate these benefits. Apart from processing conditions, starch digestion is dependent on starch properties such as granule size, architecture, crystalline pattern, degree of crystallinity, surface pores or channels, degree of polymerisation, non-starch components (e.g. proteins, tannins and phytate) and their inter-

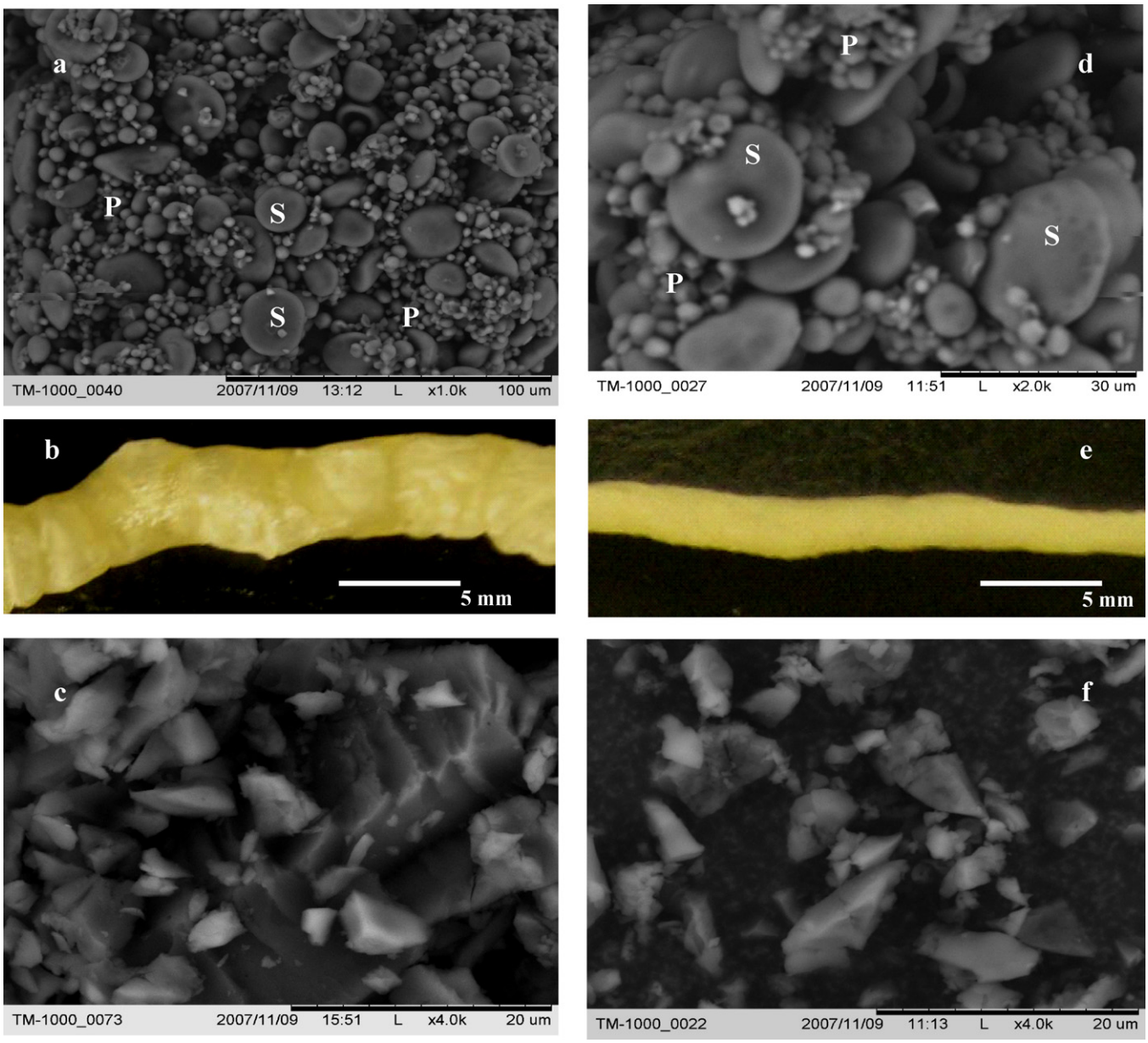

Fig. 3. Scanning electron micrographs (SEM) and photographs of typical extrudates and non-extrudates from 20% to 50% whey substitutions. (a) SEM of 20W80S non-extrudate (line = 100 μm), (b) Photograph of 20W80S extrudate (line = 5 mm), (c) SEM of 20W80S extrudate (line = 20 μm), (d) SEM of 50W50S non-extrudate (line = 30 μm), (e) Photograph of 50W50S extrudate (line = 5 mm), (f) SEM of 50W50S extrudate (line = 20 μm). Extrudates were produced at 160 °C temperature, 30% moisture, 220 rpm.

actions with starch, and amylose:amylopectin ratio (Benmoussa, Suhendra, Aboubacar, & Hamaker, 2006; Choi, Woo, Ko, & Moon, 2008; Tester, Qi, & Karkalas, 2006; Thompson, 1988; Vieira & Sarmento, 2008). During extrusion, intact starch granules might be completely lost (Fig. 3), and digestive enzymes are expected to diffuse into the processed starch and commence digestion at the contact points. This may occur instantaneously or within a short time and increase very rapidly digested starch (VRDS). But non-starch components might modify the surfaces of processed starch as well as the enzyme-substrate contact points. Judging from an increase in D_0, whey proteins possibly enhanced enzyme diffusion through favourable protein–protein interactions, surface effects or both. Moreover, pepsin treatments (gastric digestion) of the samples for 30 min prior to pancreatic digestion could have reduced any starch–protein interactions while the protein was (partially or wholly) digested to increase enzyme access to the processed starch.

In grains, for example sorghum, where starch–protein interactions limit starch digestion, Benmoussa et al. (2006) measured an increase in starch digestion after pepsin treatments of raw sorghum starch. Hence, assuming other favourable conditions, one expects factors that increase the extent of digestion to increase the rate of digestion, and vice versa. However, this might not apply in all cases possibly, if after the initial very rapid digestion, detrimental molecular or structural changes in the digesta reduce the rate of digestion. In the present study, we observed (not shown) an inverse relationship between D_0 and K, and the inverse relationship was more for 50W50S, the highest whey substitution. The reasons behind this are not immediately clear, but Chanvrier et al. (2007) and Htoon et al. (2009) opined that retrogradation-like changes that hinder digestion might occur during *in vitro* digestion because of heat and enzyme activities. However, in order to examine structural and molecular changes in the residues after *in vitro* starch

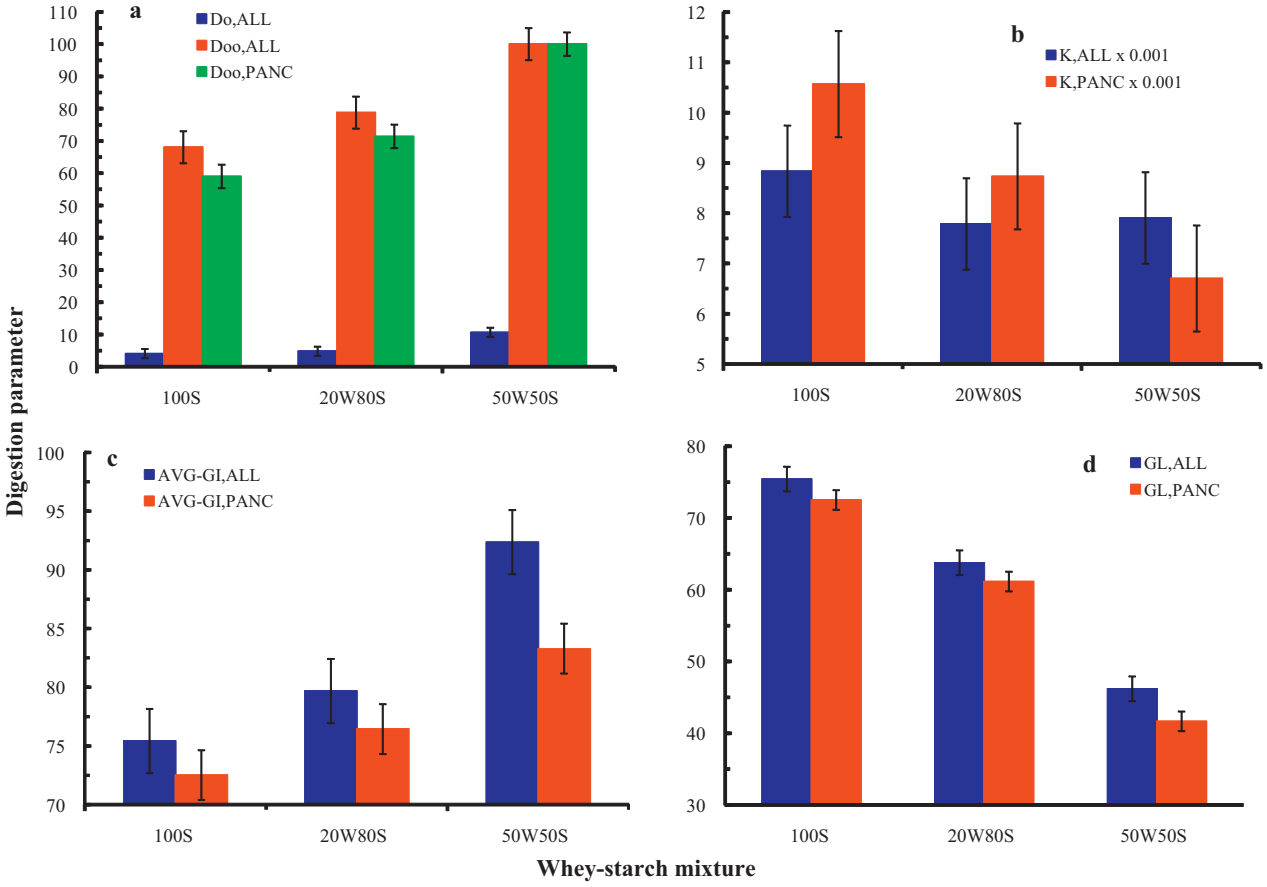

Fig. 4. Effects of whey concentration on starch digestion parameters of the non-extrudates. (a) D_0 and D_∞, (b) K, (c) AVG-GI, (d) GL. Error bars are standard errors of the means.

digestion, these authors treated the digesta with 95% ethanol to stop enzyme activities. Although the proportion of the ethanol to the total volume could have been low, it is unknown how the drying effects of ethanol impacted the structures of the residues. In the present study with about 1% solids content, it is doubtful if there could have been any major retrogradation-like changes, if at all present. We came to this conclusion because, as highlighted above, starch retrogradation is possibly minimal in dilute solutions, where starch molecules are widely dispersed to possibly hinder re-association or re-crystallisation. In addition, the presence of protein molecules would have diluted the starch–water matrix to further hinder appreciable starch–starch re-association or re-crystallisation that may limit starch digestion. Although structural and molecular changes during retrogradation are complex, deductions about retrogradation have been extrapolated from, for example, the rapid visco-analysis (RVA) studies. The setback viscosity, which is the difference between RVA peak (or trough) and final viscosities, is regarded as a direct measure of starch retrogradation (Carvalho, Onwulata, & Tomasula, 2007; Mariotti, Sinelli, Catenacci, Pagani, & Lucisano, 2009). Apart from high amylose corn starch (Hylon VII), whey protein isolate reduced the setback viscosity (minimal retrogradation) of tapioca, amioca, and corn starches (Carvalho et al., 2007), while the setback viscosity of 50W50S was significantly less than 20W80S for both whey protein concentrate and isolate (Sopade et al., 2006). It is, therefore, unlikely that retrogradation during digestion could wholly explain the inverse relationship between whey concentration and rate of starch digestion in the present study if exogenous proteins enhance digestion. However, a reduction in the rate of digestion reported in the present study supports the widely held view that whey fortification would lower the rate at which glucose appears in the blood upon consumption of HPLC foods.

On the relationship between *in vitro* rate of starch digestion and estimated glycemic index or its functions, the studies by Goñi et al. (1997) and Benmoussa et al. (2006) showed increases and decreases. Using the first-order kinetic model, Goñi et al. (1997) calculated glycemic index (GI) relative to white bread, rate of starch digestion (K) and maximum digested starch (D_∞) for 10 foods that included boiled potatoes, rice and spaghetti. In this study, boiled potatoes with a lower K than spaghetti had a higher GI, while rice with identical D_∞ as spaghetti, had a lower K and a slightly lower GI. Expressing time in min and digested starch as g glucose equivalent, we applied Eq. (3) with $D_0 = 0$ to the six digestograms of pepsin-treated and untreated raw sorghum starches from Benmoussa et al. (2006). Pepsin treatments increased K and area under the digestograms (Eq. (4)), AUC (from 0 to 720 min) for all the samples. Among the non-treated samples, a sample with the highest K gave the lowest AUC, while a pepsin-treated sample that gave the highest K, gave the highest AUC. With these examples, the rate of digestion (K) can reduce while AUC or GI increases. Apart from the basic deductions that *in vitro* GI or AUC is computed from K, D_0 and D_∞ (Eq. (4)), molecular and structural characteristics of the substrate, and possible enzyme inhibitors (Gaouar, Aymard, Zakhia, & Rios, 1997) in the digesta would influence these parameters to define effective digestibility, glycemic index and glycemic load of the foods.

From Figs. 4c and 5c, whey could increase GI of processed and non-processed HPLC foods. This is probably the first study to demonstrate this, and it might imply that (whey) protein-fortified starch snacks are not always low in GI relative to the

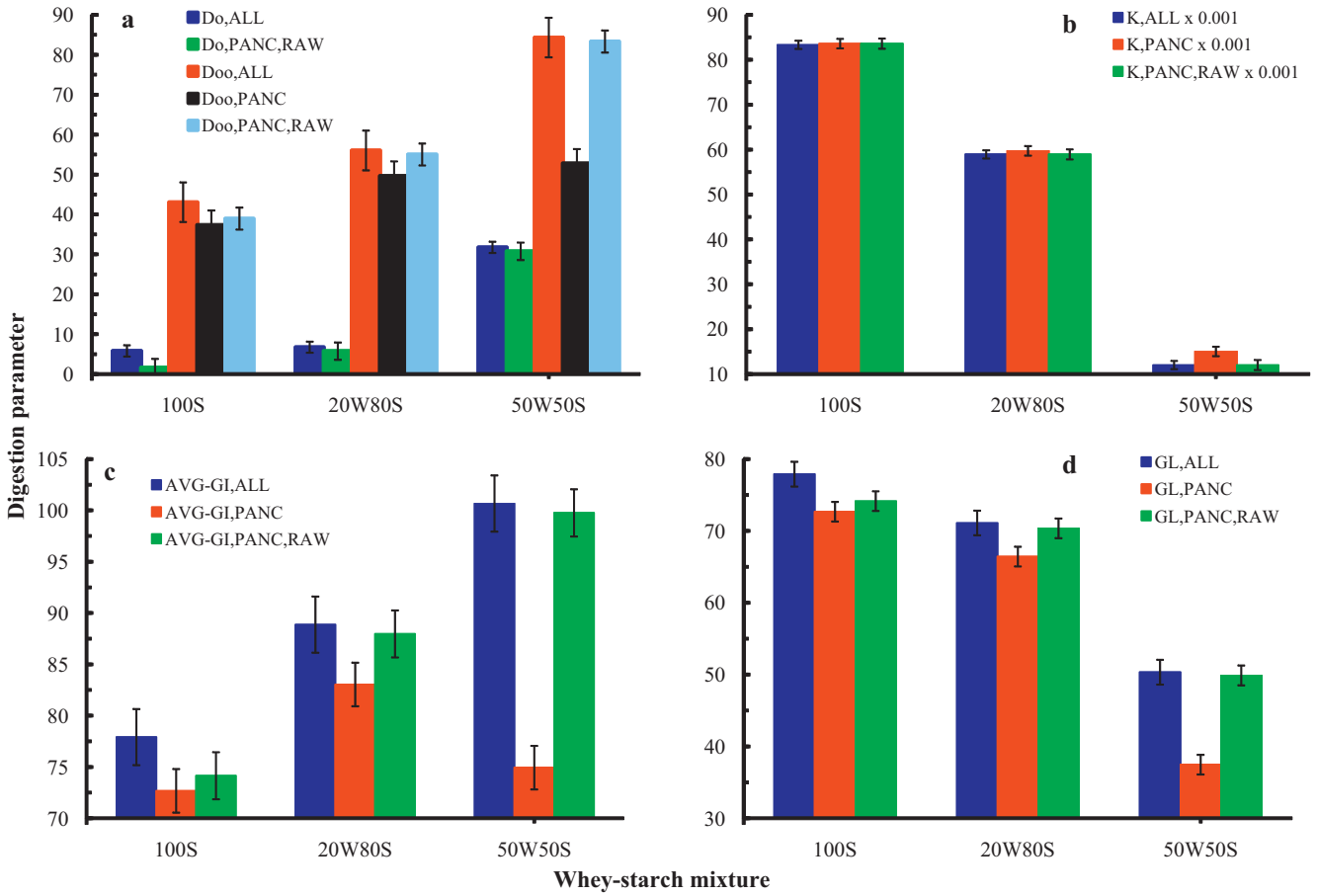

Fig. 5. Effects of whey concentration on starch digestion parameters of the extrudates produced at 50% moisture, 300 rpm and 110 °C maximum barrel temperature. (a) D_0 and D_∞, (b) K, (c) AVG-GI, (d) GL. Error bars are standard errors of the means.

non-fortified snacks. Apart from the ability of exogenous proteins in enhancing digestion, whether *in vitro* or *in vivo* starch digestion, protein fortification reduces the amount of starch to be digested per unit weight of food. In the presence of excess enzymes in test-tubes or gastrointestinal tracts, this implies that the substrate for amylolysis reduces. This could lead to more digestion (higher enzyme:substrate ratio), and an increase in glucose released. The GI might increase as a result of this, while the non-digested starch (or resistant starch) reduces within a defined digestion period. The procedure (Sopade & Gidley, 2009) used for the *in vitro* digestion in the present study used a fixed enzyme:weight ratio, thereby varying the enzyme:starch ratio for samples with different starch contents. For example, Shirani and Ganesharanee (2009) described a procedure, in which a fixed enzyme:starch ratio was used for single-point measurements. Although the procedure in the present study expressed digested starch relative to the total starch, unpublished studies in our laboratories did not reveal a consistent trend when the enzyme:starch ratio was fixed by varying the weight and/or proportion of starch in starch–non-starch mixtures. Also, some of the digestion parameters were not significantly different in these mixtures, and we believe this indicates that excess enzymes were used in the procedures by Sopade and Gidley (2009).

An increase in whey concentration, will no doubt, increase whey bioactives, but the reported increases in starch digestion shown in Table 3 and Figs. 4 and 5 might project HPLC foods as being non-beneficial to health (obesity, type 2-diabetes and gut health). There are reports that diets that are high in casein and whey could damage colonic DNA, and thin colonic mucus layer, but these cancerous effects would reduce with an increase in resistant starch (Toden, Bird, Topping, & Conlon, 2006). It would appear, therefore, that ingredients (e.g. high amylose starches) to increase resistant starch need to be a component of HPLC foods for maximum nutritional and health benefits. Or processing conditions should be chosen to increase resistant starch (e.g. RS2 and RS3). While GI as a sole parameter appears to be an incomplete parameter in comparing foods that differ in starch contents, glycemic load (GL) incorporates GI and starch content for a standard weight of food consumed (Sieri et al., 2010). Figs. 4d and 5d show GL reduced with whey concentration as the amount of starch decreased. Therefore, on the basis of GL, which is a more universal parameter, whey fortification is beneficial. Hence, the benefits of HPLC foods, and possibly fibre-enriched foods, might be better emphasised on the basis of low GL, because (*in vitro*) GI might not be low in all the formulations.

4. Conclusions

Whey proteins increase the protein and lower starch contents of starch-based snack foods, and their functional properties make them ideal for fortifying such foods. The present study showed that although glycemic load significantly ($p < 0.05$) decreased with whey substitution, starch digestibility and glycemic index of whey-fortified foods can increase. This will reduce resistant starch and limits the health benefits of high-protein-low-carbohydrate foods, even though whey bioactives in such foods can be substantial. Hence, processing of whey-fortified snacks needs to increase resistant starch by a careful choice of processing and storage conditions. Alternatively, starches that are high in amylose content, known for their resistance to digestion, ought to be a component of

the ingredients to boost the content of resistant starch in the finished product. Also, whey proteins can aid retrogradation in high-amylose starches (Carvalho et al., 2007) to increase resistant starch type 3 (RS3). The present study revealed that whey substitution up to 50% can produce directly expanded extrudates, but expansion and resistant starch could be maximised with a 20% whey formulation, and possibly extruded at 160 °C, 50% moisture and 300 rpm using the extruder (and screw configuration) described in the present study. The specific mechanical energy from such extrusion conditions would be moderate (less than 200 kJ kg^{-1}). Although this is the first report on the kinetics of starch digestion in whey–starch systems, optimum conditions for processing of whey–starch mixtures are not solely determined by starch digestibility. Protein digestibility and stability of whey bioactives in the processed whey–starch mixtures are of immense importance, and future papers from our laboratories are to address these issues.

Acknowledgement

The authors are grateful to Dr Olena Kravchuk of the School of Land, Crop and Food Sciences, the University of Queensland, St Lucia, QLD 4072, Australia, for statistical assistance.

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