Kinetics of starch digestion in Australian sweetpotato as affected by particle size

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Abstract

In root crops, starch is the major component and main energy fraction. Recent trends in human nutrition with respect to health concerns (e.g. obesity and diabetes) have focused on starch digestion to identify digested and resistant starches. Knowledge of digestion kinetics helps to understand the contributions of these components, as well as the factors influencing them. We investigated the kinetics of starch digestion in the *Beauregard* cultivar of sweetpotato as affected by grinding types and conditions. The cultivar was peeled, diced, sulphited, and hot-air dried prior to cryo-milling and hammer milling at 11 different conditions to a range of particle sizes (80 – 380 μm) in a completely randomized replicated design. Time-course *in-vitro* starch digestion was studied using artificial saliva, pepsin, pancreatin, and amyloglucosidase, and digested starch was measured by glucometry. All the samples essentially exhibited monophasic digestograms, which were described using a modified first-order model. Grinding conditions affected ($p<0.05$) the particle size, and subsequently the initial or very-rapidly digested starch and rate of digestion (K, s^{-1}) because of changes in effective surface area. Significant linear relationships were obtained between the reciprocal of the rate of digestion (1/K, s) and the square of the average particle size ($size^2$) for both mills separately and combined. The reciprocal of the slope (2.1 – 3.7 x 10^{-7} cm^2s^{-1}) revealed digestion proceeded by diffusion mechanisms. The likelihood of heat generation during hammer milling appeared to influence starch digestion. The digestion parameters of the sweetpotato are discussed with other materials (e.g. cereals), and related to starch properties.

Keywords: *In-vitro* starch digestion, Monophasic digestogram, Diffusion mechanisms, First-order kinetics

Introduction

Sweetpotato is growing in importance in Australia because of its potential health benefits (vitamins and antioxidants). An estimated 40,000 MT was produced in Australia in 2006, with *Beauregard, Northern Star* and *Kestle* as the main commercial cultivars (Maltby *et al.*, 2006). Sweetpotato is a starchy crop (Wolf, 1992), and generally, the modes and patterns of enzymatic digestion of starch define the health and nutritional significance of the food. With the existence of pores or channels in some starch granules, the interlinked stages in enzyme digestion of starch (amylolysis) are thought (Benmoussa *et al.*, 2006; Tester *et al.*, 2006; Sopade *et al.*, 2008) to be (i) random diffusion of enzymes onto the surface of starch granules, (ii) random movement of part of enzyme by capillary action to inside the granules, (iii) amylolysis commences at these points, (iv) amylolysis proceeds radially (centripetal) to create additionally pores and channels to the core of granules, (v) amylolysis proceeds and spreads from the core by enzymes inside the granule (centrifugal). In root crops, enzyme digestion of starch is thought to be mainly by the centrifugal mode (Tester *et al.*, 2006), but it seems the centripetal mode possibly needs to establish the route to the core of starch granules before centrifugal mode can become prominent (Sopade *et al.*, 2008). While these stages appear to mainly apply to extracted pure starches, the situation with starches existing in complete food systems is not as well established particularly in relations to enzyme diffusion.

Using milled sorghum grains, Mahasukhonthachat *et al.* (2009) proposed that starch digestion in sorghum proceeded by diffusion mechanisms by investigating the rate of *in-vitro* starch digestion at different particle sizes. We are not aware of any studies done on root crops, and specifically on sweetpotato. The particle size of food can be reduced (Fellows, 1998) by using various mills (e.g. cryomilling, hammer milling, roller milling, and attrition milling). These mills not only vary in their predominant grinding mechanisms, the extent of frictional heat can differ. The grinding mechanisms (e.g. impact, attrition and shearing) and frictional heat can bring about diverse structural and molecular changes, and affect the functional and digestibility properties of the food. While impact force (mechanical effects) is common to both cryo- and hammer-milling, cryomilling freezes materials in liquid nitrogen to their glassy state prior to grinding in liquid nitrogen. This effectively eliminates frictional heat, which can be substantial in hammer milling. Hence, we expect both cryo- and hammer-milling to influence functional properties of food differently, as well as the modes and patterns of starch digestion. Therefore, this study investigated how cryo- and hammer-mills affected the mechanisms of starch digestion in non-processed sweetpotato, and examined the dependence of starch digestion on possible frictional heat during hammer milling.

Materials and methods

Materials

Fresh tuber. The sweetpotato tubers (cultivar *Beauregard*) used were obtained from the Queensland Department of Primary Industries and Fisheries, Gatton QLD 4343. The skin colour was orange, the flesh colour was pale orange, while the cortex was thin, and the tuber shape was obovate (CIP, AVRDC and IBPGR, 1991). The L* a* b* indices of the flesh were 60.2, 22.6 and 34.9 respectively (white tile, L* = 97.1, a* = 0.1, b* = 1.9), while the moisture content (%) was 80 $\pm$ 0.3, total starch was 60 $\pm$ 2.8 g/100g dry solids and the peel was about 6%.

Method

Sweetpotato drying. After washing and peeling, the tubers were diced (3.5 mm cutting disc, 7 mm cutting blade, 1420 rpm; Serial 7-897, Hallde RG-7, 26 Kista, SE-164, Sweden) and soaked in a 0.3% sodium metabisulphite solution for 5 min. before air-dried (TD-36T-1-D, Thermoline Scientific Pty Ltd, Smithfield, NSW 2164, Australia) at 40°C and about 0.8 m/s for 4 days. The dried dices were packed in polythene bags and cool-stored before further processing and analysis. The solids yield (total weight of solids in the dried dices) was about 72% of the total weight of solids in the fresh tubers.

Moisture content. Moisture content was determined according to AOAC Method 2.2.01 (AOAC, 1995); oven drying at 100°C for 24 hr (constant weight).

Flour milling. The dried dices were cryomilled (6850 SPEX Freezer/Mill; SPEX, Metuchen, NJ 08840, USA) and hammer-milled (MFC type DCFH 48, John Morris Scientific Pty. Ltd., Eagle Farm QLD 4009, Australia) using 11 different settings to vary the particle size of the sweetpotato flour. The milling was replicated, and the sweetpotato flours were stored in air-tight plastic bottles until analysed.

Particle size analysis. The Malvern Mastersizer Hydro 2000MU (Malvern Instruments Ltd, Malvern WR14 1XZ, UK) was used to analyse the particle size of the flours in water at 2000 rpm. A general purpose analysis model was used with particle refractive and absorption indices of 1.52 and 0.1 respectively, while the refractive index of water as the dispersant was 1.33. Particle size (v/v, μm) was mainly defined as the volume weighted mean (d[4,3]), and particle sizes of the 10^{th}, 50^{th} (median) and 90^{th} percentiles were used in addition to characterize and define the size distribution. However, discussions on the effects of particle size will concentrate on the volume weighted mean, henceforth referred to as the average particle size.

Total starch analysis. The total starch content was determined using a method derived from Megazyme (Megazyme International Ireland Ltd., Wicklow, Ireland) based on dimethyl sulphoxide (DMSO), α-amylase (AA) and amyloglucosidase (AMG) procedure (Mahasukhonthachat *et al.*, 2009).

In-vitro starch digestion. The time-course starch digestion was analysed using a rapid *in-vitro* procedure (Sopade and Gidley, 2009; Mahasukhonthachat *et al*; 2009) that involved digesting about 0.5 g of ground sample with amylase, pectin, pancreatin, and amyloglucosidase in appropriate buffers and pH, and periodically measuring the glucose produced by a glucometer up to 4 hr.

Statistical análisis. General linear model analyses (Minitab®, release 15; Minitab Inc.) were conducted, and the significance level for individual tests was 5%. The p-values of tests are reported, as well as the 95%-least significance differences (LSD). Wherever applicable, samples were randomized and, at least, duplicated for all the physicochemical analyses described above.

Results and discussion

Particle size analysis

The 11 different settings on the cryo- and hammer-mills yielded sweetpotato flours of different particle size parameters (Table 1), and all the flours exhibited essentially bimodal size distributions (Fig. 1). The mill settings or grinding conditions significantly ($p<0.05$) affected the average particle size. It can be observed in Table 1 that, generally:

- Finer sweetpotato flour was obtained when the cryomilling cycles were increased. This agrees with the results on sorghum grains (Mahasukhonthachat *et al.*, 2009), and follows from increased milling time and intensity.
- Coarser sweetpotato flour was obtained when the aperture of the retention sieve in the hammer mill was increased. This is expected because of less resistance and reduced residence time in the action zone as the sieve size increased.
- With more passes, finer sweetpotato flour was produced as more particles were further broken down.

Table 1. Milling conditions for the sweetpotato flour and the particle size parameters[a]

Sample code	Milling condition		Particle size parameter (v/v, μm)[a]							
			d(v,0.1)[b]		d(v,0.5)[b]		d(v,0.9)[b]		Volume weighted mean d(4,3)	
Cryomilling										
	Grinding time (min) per cycle	**Number of cycle**								
CG1	5 [30]c	6	7•	0.5	28•	2.7	237•	15.3	82	6.1
CG2	5 [10]c	2	8•	0.5	84•	5.8	474•	10.0	171	4.9
CG3	5[5]c	1	11•	0.2	165•	14.5	741•	31.1	283	15.9
CG4	5[15]c	3	7•	0.2	53•	5.3	378•	23.8	132	8.5
CG5	5[20]c	4	7•	0.1	36•	0.5	306•	3.6	105	0.4
Hammer milling										
	Retention sieve (mm)	**Number of regrind**								
HM1	2	1	13•	0.4	200•	6.7	1016•	62.2	376	20.1
HM2	2	3	11•	0.1	97•	5.1	742•	9.7	256	1.2
HM3	2	6	10•	0.1	77•	2.4	675•	36.2	226	9.7
HM4	1.5	1	10•	0.4	62•	15.8	782•	16.7	256	11.8
HM5	1	1	10•	0.2	60•	0.5	782•	14.2	255	4.2
HM6	0.5	1	8•	0.1	26•	0.8	426•	5.5	119	1.2

[a] Figures are means ± standard deviations.
[b] These indicate 10^{th}, 50^{th} (median) and 90^{th} percentiles
[c] Figures in [ ] show the total grinding time. The same pre-cool time (5 min.), intermediate cooling time (2 min.) and impactor speed ($10\ s^{-1}$) were used for cryomilling.

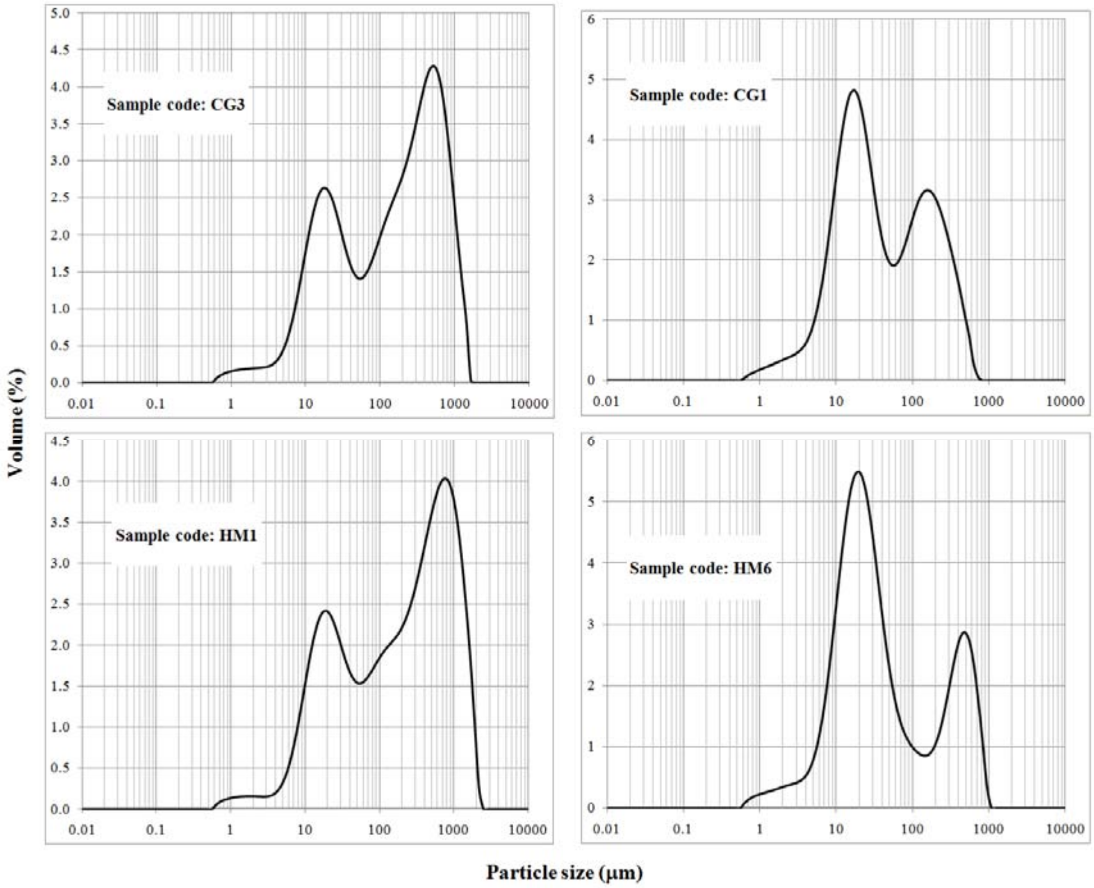

Figure 1. Typical particle size distribution of the cryo-(CG) and hammer-(HM) milled sweetpotato flour

From published studies (Kerr *et al.*, 2000; Huang *et al.*, 2008; Mahasukhonthachat *et al.*, 2009), in addition to reducing the particle size, milling time and intensity lead to structural and molecular changes, which affect functional and digestibility properties of starch.

In-vitro s*tarch digestión.* Irrespective of the milling conditions, and consequently the average particle size, starch digestion in the sweetpotato flours exhibited a monophasic digestogram, and significantly ($p<0.05$) increased with time in a non-linear manner (Fig. 2). The time effect is consistent with an increase in reaction products with time of reaction, and agrees with other studies (Goñi *et al.*, 1997; Sun *et al.*, 2006). Although monophasic digestograms are popular in food systems, the monophasic digestion pattern of the cryo- and hammer-milled sweetpotato flours is contrary to the biphasic pattern obtained with sweetpotato from Papua New Guinea (Liu *et al.*, 2009). We associate this to differences in genotype and environment (G x E), two factors that are well known to affect properties of plant materials. With special reference to digestibility, G x E can affect the rate and extent of digestion, and might influence the dominant digestion mechanisms. The rate and extent of digestion are better quantified by modeling the digestograms to yield parameters and understand the mechanisms of digestion.

***Modeling* in-vitro *starch digestión*.** Both empirical and theoretical models are widely used in describing digestograms, and of particular importance is the first-order exponential model (Goñi *et al.*, 1997). This model has been modified (Mahasukhonthachat *et al.*, 2009) to include a parameter for the initial digested starch, very rapidly digested starch or a measure of *in-vitro* gastric digestion.

$$D_t = D_o + D_{\infty\text{-}o}\,(1 - \exp[-K\,t]) \qquad (1)$$

$$D_\infty = D_o + D_{\infty\text{-}o} \qquad (2)$$

where, D_t = digested starch at time t (g/100g dry starch), D_o = digested starch at time t = 0 (g/100g dry starch), D_∞ = digested starch at infinite time, $t \rightarrow \infty$ (g/100g dry starch), K = rate of digestion (min^{-1}). Using the Microsoft Excel Solver™, and constraining $D_o \geq 0$, and $D_\infty \leq 100$, Eqn.[1] adequately described (r^2 = 0.981 – 0.999) the digestograms for the cryo- and hammer-milled sweetpotato flours (Fig. 2). Although the parameters of the model are significantly ($p<0.05$) dependent on the grinding conditions, and hence, the particle size of the flour, the present focus is on the dependence of the rate of digestion on particle size. Generally, the higher the particle size, the lower was the rate of digestion, irrespective of the mill. This is consistent with a reduction in available surface area for enzymatic reactions as size reduces.

The surface area of a spherical particle is directly proportional to the square of the radius or diameter of the particle. If an enzyme diffuses onto the surfaces of spherical particles, and digestion proceeds by first-order kinetics, the rate of digestion is inversely proportional to the square of the particle size ($1/K = C \times size^2$, where C = constant of proportionality and a measure of coefficient of diffusion). Hence, a plot of the reciprocal of K against the square of the particle size is linear with a zero intercept, but deviations from the simplified theory are compensated for with an intercept. With or without combining the data from the two mills, starch digestion in the sweetpotato flours followed this simplified diffusion theory (Fig. 3). The reciprocal of the slope equates to the apparent diffusion coefficient (D_{iff}), and from Smoluchowski analysis, diffusion mechanisms prevail if the apparent diffusion coefficient is of the order of 10^{-7} cm^2 s^{-1}.

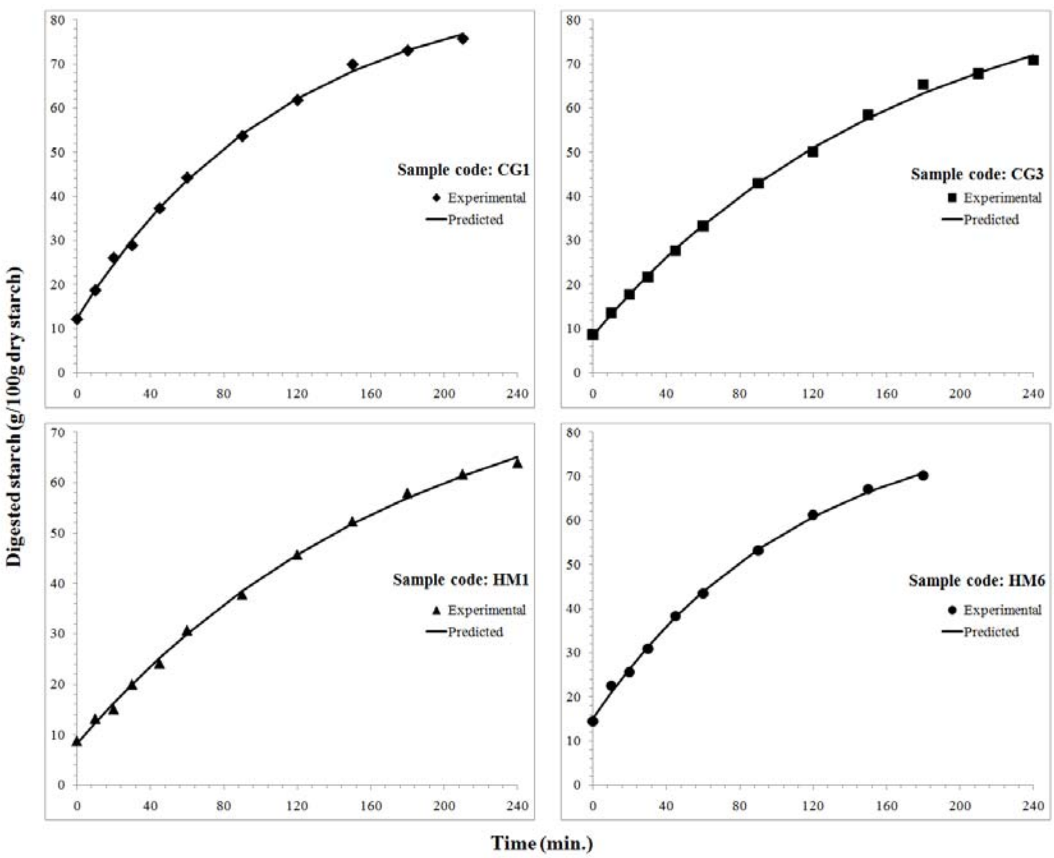

Figure 2. Typical monophasic digestogram (experimental and predicted) of the cryo-(CG) and hammer-(HM) milled sweetpotato flour

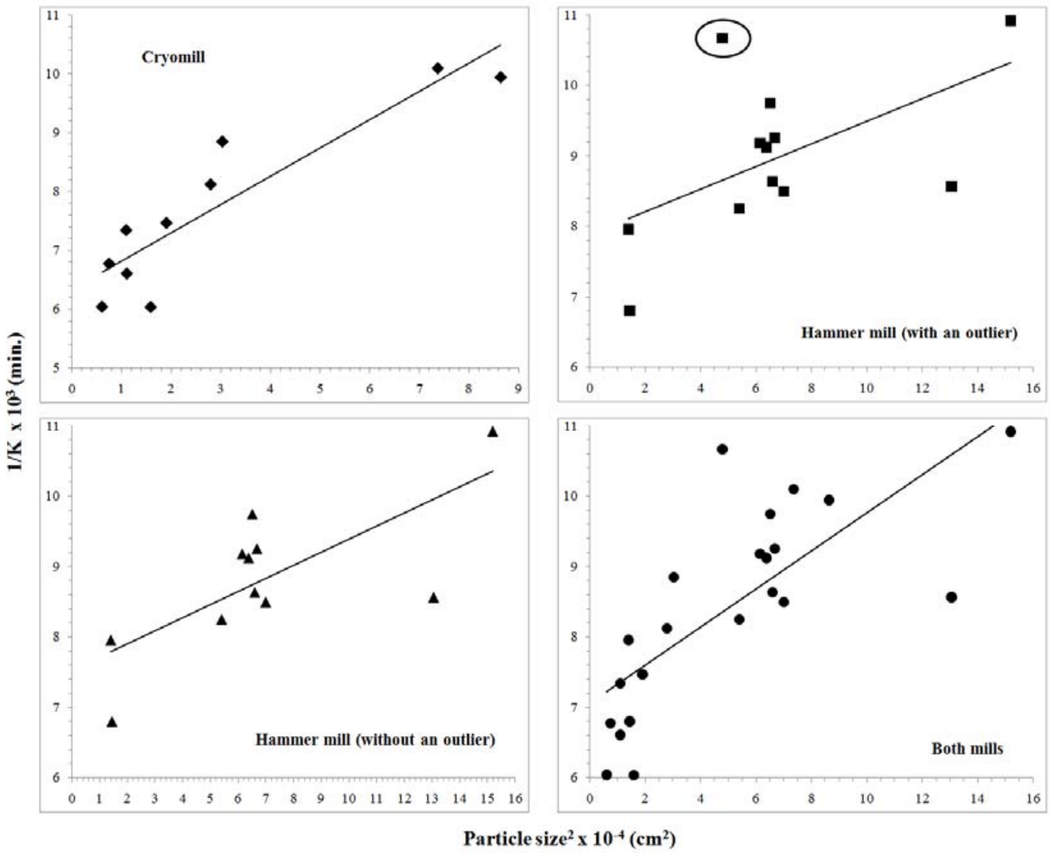

Figure 3. The relationship between the reciprocal of the rate constant and square of the particle size for the sweetpotato flour

Table 2 shows the equations relating the rate of digestion with the square of particle size for the sweetpotato flours, and it can be inferred that starch digestion by amylases in the cryo-milled and hammer-milled sweetpotato flours proceeded by diffusion mechanisms. In an earlier study (Mahasukhonthachat *et al.*, 2009), diffusion mechanisms were also inferred to prevail during starch digestion of sorghum. Although sorghum is a cereal, and sweetpotato is a root crop, the similarity between the prevailing mechanisms during amylolysis could be because both starches have A-crystalline pattern. However, from the apparent diffusion coefficients (D_{iff}) in the milled sorghum (0.4 – 0.9 x 10^{-7} cm^2 s^{-1}) and sweetpotato flours (2.1 – 3.7 x 10^{-7} cm^2 s^{-1}), digestion was faster in the sweetpotato flours. Generally, sweetpotato starch (2 – 72 μm) is bigger than sorghum starch (5 – 30 μm) to suggest sorghum would be easier to digest (Sopade *et al.*, 2008). However, unlike sweetpotato, starch-protein interactions are pronounced in sorghum, they limit starch digestibility, and possibly reduce apparent diffusion coefficient of amylases to sorghum starch.

Table 2: Relationship between the square of the particle size and the reciprocal of the rate constant

Mill*	Equation	r^2	D_{iff} (cm^2 s^{-1}) x 10^{-7}
CG	1/K = 4.8 x 10^6 $(size)^2$ + 7.1 x 10^3	0.826; p<0.001	2.1
HM	1/K = 1.6 x 10^6 $(size)^2$ + 7.1 x 10^3	0.318; p=0.035	6.2
HM (without outlier)	1/K = 1.9 x 10^6 $(size)^2$ + 6.9 x 10^3	0.533; p=0.005	5.3
Both mills	1/K = 2.7 x 10^6 $(size)^2$ + 6.9 x 10^3	0.562; p<0.001	3.7

*CG = Cryo mill, HM = Hammer mill; One HM outlier with standardized residual of 2.5

Although not clearly defined, there were slight but measureable differences in the digestibility of the hammer-milled and cryo-milled sweetpotato flours. This demands further investigations as it appears the hammer-milled flours were easier to digest because the apparent diffusion coefficients of the hammer-milled flours are numerically higher (Table 2) than those of the cryo-milled flours. The slight difference could be because of frictional heat during hammer milling, and/or differences in the mode of size reduction. We measured increases in flour temperatures (5 – 13°C) during the hammer milling to suggest more heat was generated as the diameter of the retention sieve reduced. Hammer milling was suspected to increase the digestibility of sorghum grains in comparison with cryomilling, and differences in the functional properties of hammer- and cryo-milled sorghum flours were reported ((Mahasukhonthachat *et al*., 2009). Therefore, additional studies on the functional properties (pasting, water absorption, water solubility, gelatinisation, and spectroscopy) of the sweetpotato flours, in progress in our laboratories, might help establish the suspected differences, if any, and if they are measureable in these properties. Moreover, it is worth stressing that even though both the cryo- and hammer-milled sweetpotato flours showed essentially bimodal particle size distributions (Fig. 2), differences could exist between the proportions of small to large particles within and between the mills. In addition to the 10^{th}, 50^{th} and 90^{th} percentiles (Table 1), an approach under investigation in our laboratories is to deconvolute the size distribution curves, and examine the contributions of small and large particles to gastric and pancreatic digestions. This could assist in understanding if, and to what extent, frictional heat during the hammer milling affected starch digestibility in the sweetpotato flours, and the consequences of this to the overall utilisation of sweetpotato as a functional food, and in specialised foods.

Conclusions

Starch digestion in the *Beauregard* cultivar of Australia sweetpotato proceeded by diffusion mechanisms, and more starch was digested when the particle size was reduced. *In-vitro* pancreatic digestion of the flours was time-dependent in a non-linear manner. The conditions in the cryo- and hammer-mills significantly affected the particle size of the sweetpotato flours, and there were slight but measurable effects of mill type on starch digestibility. Differences in the mechanical action and frictional heat during milling were suspected to be responsible for the differences in the mills, but this demands further investigations. Future studies will investigate the contributions of small and large particles on the diffusion mechanisms, and generally how particle size influences functional properties of the flours. The importance of the particle size-digestibility studies lies in understanding the optimum size and distribution for starch digestibility of specialized products from sweetpotato.

Acknowledgement

The authors are grateful to Ms Sandra Dennien of the Queensland Department of Primary Industries and Fisheries, Gatton for supplying the sweetpotato tubers.

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