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Relationship between granule size and *in vitro* digestibility of maize and potato starches

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ABSTRACT

Maize and potato starch granules were separated by a sedimentation method into a range of fractions based on their size. The surface weighted mean diameter [$D(3,2)$] of separated granules was in good agreement with predictions from Stokes' law of sedimentation. *In vitro* digestion of fractionated starches by α-amylase was well fitted by first-order kinetics, with the digestion rate coefficient (K) showing an inverse square relation with granule size, consistent with either a diffusion-controlled or surface-controlled mechanism. Apparent diffusion coefficients of α-amylase obtained by fitting the size dependence were 7.40 (maize starch) and 1.35 (potato starch) $\times 10^{-10}$ cm^2 s^{-1} respectively. A correlation between K and specific granule surface area was also obtained for both starches, consistent with a role for surface area in controlling amylase digestion rates. Differences in K values are consistent with electron microscopy of partially digested granules, suggesting that an external surface-controlled mechanism may be operating for potato starch, and that the effective surface area of maize starch is greater than predicted from granule diameter due to surface pores and channels.

1. Introduction

In nature, starch exists as granules with varying sizes (~1–100 μm in diameter), shapes (spherical, lenticular, polyhedral and irregular), size distributions (unimodal and bimodal), and in forms (simple and compound) characteristic of their botanical origin (Tester, Karkalas, & Qi, 2004). The enzymatic hydrolysis of native starch granule is affected by a range of factors including granular structure (shape, size and porosity), supramolecular structure (organisation of growth rings and degree of crystallinity), molecular structure (fine structure of amylose and amylopectin), presence of non-starch materials, reaction conditions and enzyme specificity (Buleon, Colonna, Planchot, & Ball, 1998; Svihus, Uhlen, & Harstad, 2005; Yook & Robyt, 2002). The effect of granule size on enzymatic susceptibility of starch has been investigated by various authors (Franco & Ciacco, 1992; Franco, Ciacco, & Tavares, 1998; Kasemwong, Piyachomkwan, Wansuksri, & Sriroth, 2008; Noda et al., 2005; Sahal & Jackson, 1996) with the general and unsurprising result that smaller granules tend to hydrolyse faster than larger ones, for each of several botanical sources. The hydrolysis process includes the diffusion of enzymes to the granule surface followed by adsorption and subsequent catalytic events (Colonna, Leloup, & Buleon, 1992). Smaller granules, by virtue of their higher available surface area per unit mass, facilitate diffusion and adsorption of enzymes, accelerating the catalytic action. The diffusion-controlled mechanism of enzymatic hydrolysis has also been studied in milled sorghum and barley particles (Al-Rabadi, Gilbert, & Gidley, 2009; Mahasukhonthachat, Sopade, & Gidley, 2010) where diffusion of amylase through the dense endosperm matrix to starch granules is proposed to be rate-determining. However, so far, no work has been done to relate the effect of granule size or surface area to amylase reaction kinetics in major commercial starches such as maize and potato. One of the limitations of such studies is the requirement for size separation techniques that give narrow particle size fractions. Sedimentation (Ao & Jane, 2007; Sahal & Jackson, 1996; Takeda, Takeda, Mizukami, & Hanashiro, 1999) and sieving (Peng, Gao, Abdel-Aal, Hucl, & Chibbar, 1999; Yamazaki & Wilson, 1964) are widely used in size separation of starches. The major shortcoming of sedimentation techniques has been the assumption of spherical granules of uniform density (Rasper, 1971). In sieving methods, spherical or polygonal starch granules are separated at the measured mesh size, but those with other shapes are not well separated (Yamazaki & Wilson, 1964). Aggregation of granules fur-

Abbreviations: K, digestion rate coefficient (min^{-1} or s^{-1}); MS, maize starch; PS, potato starch; η, viscosity (Pa s); h, sedimentation height (m); g, acceleration due to gravity (m/s^2); ρ_s, density of starch (kg/m^3); ρ_w, density of water (kg/m^3); X, particle diameter (μm, cm or m); VS, very small; S, small; M, medium; L, large; VL, very large; opm, oscillation per minute; C, starch digested (hydrolysed) (%db); t, digestion or incubation time (s or min); K_{MS}, digestion rate coefficients (s^{-1} or min^{-1}) for MS; K_{PS}, digestion rate coefficients (s^{-1} or min^{-1}) for PS; D, diffusivity coefficient (cm^2 s^{-1}); R^2, coefficient of determination.

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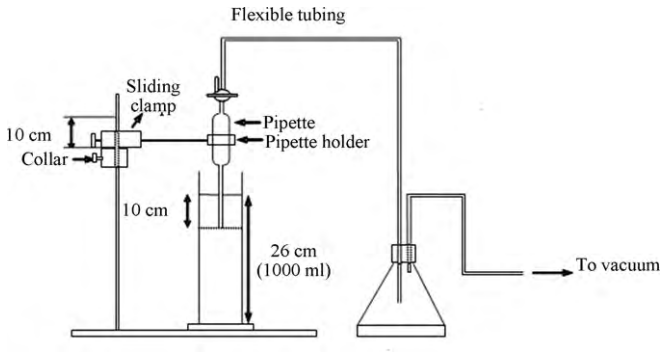

Fig. 1. Schematic diagram of the sedimentation separation of starch granules.

ther adds to the error in sieving separation. Starches of the *Triticeae* family (wheat, barley, rye and triticale) are bimodal in size distribution consisting of larger A-type and smaller B-type granules that are biosynthesised at different stages of kernel development. These granules can be separated easily, but will not provide a range of sizes for the study of enzymatic hydrolysis. In addition, compositional properties of these granules are different (Ao & Jane, 2007; Salman et al., 2009; Takeda et al., 1999) and may affect the rate and pattern of enzymatic hydrolysis. Fractionation of 'unimodal' starches like maize and potato could be a good model for the study of the effect of granule size on amylase digestibility.

In this study, a simple instrumental setup was created for size separation based on Stokes' law of sedimentation. Two commercial 'unimodal' starches, maize and potato, were fractionated into various sizes and analysed for *in vitro* amylase digestibility. Data were fitted using first-order kinetics in order to calculate rate coefficients as a function of granule size for both starches. The resulting size dependencies were analysed in terms of both (enzyme) diffusion-controlled and (granule) surface-controlled reaction mechanisms.

2. Experimental

2.1. Materials

Potato starch (S-4251) (PS) was purchased from Sigma–Aldrich Pty Ltd., Sydney, Australia and regular maize starch (MS) was purchased from Penford Australia Ltd., Sydney, Australia. The average apparent amylose contents of PS and MS, determined by an iodine colorimetric method (Hoover & Ratnayake, 2005), were 36.8% and 27.1% respectively.

2.2. Size separation of starches

Starch granules were separated into different size fractions based on the 'Pipet' method (Gee & Bauder, 1986) using the instrumental setup shown in Fig. 1. The method involved homogenization of starch in water, allowing the contents to settle for defined times, and removal by pipetting of the starch suspension that remained above a depth h, after time t, such that particles larger than X (Eq. (1)) are sedimented. The sedimentation time t (s) derived from Stokes' law is given by Eq. (1):

$$t = \frac{18\eta h}{g(\rho_s - \rho_w)X^2} \quad (1)$$

where η is the viscosity of water (1.003×10^{-3} Pa s at 20 °C), h the sedimentation height (m), g the acceleration due to gravity (9.8 m/s^2), ρ_s the density of starch (1500 kg/m^3), ρ_w the density of water (998.23 kg/m^3) and X is the particle diameter (m) (Buleon et al., 1998; Gee & Bauder, 1986).

The details of this size separation procedure are described below. Starch (20 g) was placed in a plastic cylinder and made up to 1 L (marked point) with distilled water (20 °C). The suspension was thoroughly shaken by hand stirrer for 30 s using up-and-down motions and then left undisturbed for specific time periods (as determined from Eq. (1)). After the desired time, the pipette was gently lowered (movement fixed by collar) and the top 10 cm of the suspension was vacuum suctioned into a conical flask. The cylinder was than refilled with water to the 1 L mark and shaken as previously. The process was repeated until the top 10 cm layer was clear without visible starch particles (minimum 7 repetitions required). The collected suspension was left undisturbed to settle and was recovered by suction and decanting. The starch slurry was dried in a vacuum oven at 37 °C for 48 h, and the dried starch (~6% moisture) samples were kept in sterile plastic containers and transferred to a desiccator containing silica gel. MS was separated into four fractions, viz., <10, 10–15, 15–20 and >20 μm (predictions based on Stokes law) hereafter denoted as very small (MS-VS), small (MS-S), medium (MS-M) and large (MS-L) granules respectively. PS was separated into five fractions, viz., <20, 20–32, 32–45, 45–55 and >55 μm, hereafter denoted as very small (PS-VS), small (PS-S), medium (PS-M), large (PS-L) and very large (PS-VL) granules. The percentage yields of each fraction from both starches are shown in Table 1.

2.3. Granule size analysis

Granule size analysis was carried out using a Malvern Mastersizer Hydro 2000MU (Malvern Instruments Ltd., Malvern WR14 1XZ, UK). The samples were suspended in water and stirred at 2000 rpm. A general purpose analysis model was used with particle refractive and absorption indices of 1.53 and 0.1 respectively, while the refractive index of water as the dispersant was 1.33. The obscuration in all the measurements ranged from 9% to 13%. Particle size is defined in terms of 10th percentile [$d(0.1)$], median [$d(0.5)$], 90th percentile [$d(0.9)$] and surface weighted mean [$D(3, 2)$]. The average granule size was expressed as the surface weighted mean value, i.e. the diameter of a sphere that has the same volume/surface area ratio. This was used to determine the specific surface area (m^2/g) assuming spherical granules of uniform density.

2.4. In vitro digestibility of starches

The *in vitro* starch digestion method used was adapted, with modifications, from the method described by Htoon et al. (2009). About 500 mg of starch was accurately weighed and treated with 1 mL of artificial saliva containing porcine α-amylase (250 U per mL of carbonate buffer, pH 7) for 15–20 s before 5 mL of pepsin (1 mg per mL in 0.02 M HCl, pH 2) was added and incubated at 37 °C for 30 min (simulated gastric digestion) in a reciprocating (85 oscillation per minute (opm)) water bath (SWB20; Ratek Instruments Pty. Ltd., Boronia, VIC 3155, Australia). The digesta was neutralised (5 mL, 0.02 M NaOH) followed by addition of 0.2 M sodium acetate buffer (pH 6; 25 mL) and 5 mL of a mixture of pancreatin (2 mg per mL) and amyloglucosidase (28 U per mL). The mixture was incubated (37 °C, 85 opm) for 0, 20, 120, 240, 360 and 480 min for both starches (and 1440 min for PS). After the completion of each designated incubation time, glass flasks were placed in an ice water bath for 10 min to stop enzyme activity and frozen. For subsequent glucose analysis, the flasks were thawed, contents were transferred into a 50 mL polypropylene centrifuge tubes and the volume made up with deionized water. The mixture was centrifuged at 2000 × g for 10 min and the glucose concentration in the supernatant was determined by using a glucose oxidase colorimetric analysis kit (TR-1511-200 Thermo Electron Noble Park, Victoria, Australia) with detection at 505 nm (Pharmacia LKB-Ultrospec III, England). The

Table 1
Size distribution of fractionated MS and PS.

Description	Predicted size range[a]	Sedimentation time (min)	Relative yield (%)	Malvern Mastersizer data				
				$d(0.1)$ (μm)	$d(0.5)$ (μm)	$d(0.9)$ (μm)	$D(3,2)$ (μm)	Specific surface area (m^2/g)[b]
MS	–	–	–	9.35	14.4	21.94	13.61	0.29
MS-VS	MS < 10 μm	61.2	17.2	6.37	9.72	14.57	9.18	0.43
MS-S	MS 10–15 μm	27.3	31.4	9.79	14.20	20.41	13.61	0.29
MS-M	MS 15–20 μm	15.3	40.6	12.01	17.24	24.57	16.56	0.24
MS-L	MS > 20 μm	SR[c]	10.8	13.60	20.22	29.94	20.30	0.19
PS	–	–	–	22.11	42.43	74.34	30.47	0.13
PS-VS	PS < 20 μm	15.3	12.2	11.04	16.86	25.44	15.96	0.25
PS-S	PS 20–32 μm	5.98	26.4	20.41	29.26	41.77	28.12	0.14
PS-M	PS 32–45 μm	3.02	32.5	29.44	41.79	59.44	40.26	0.10
PS-L	PS 45–55 μm	2.02	19.8	37.16	52.39	73.60	50.53	0.08
PS-VL	PS > 55 μm	SR[c]	9.1	50.03	69.86	97.37	67.54	0.06

[a] Size based on Stokes law of sedimentation.
[b] Based on the assumption of spherical granules of density 1500 kg/m^3.
[c] Sediment remaining after predecessor fraction was removed.

value was multiplied by a factor of 0.9 to convert glucose concentration into starch with results presented as gram per 100 g dry starch. All the measurements were carried out with two replicates and results are expressed as means ± standard deviation of replicates.

2.5. Data fitting

The standard first-order rate equation (Eq. (2)) was used to investigate the kinetics of starch digestion under simulated small intestine conditions by α-amylase.

$$C = 1 - e^{-Kt} \quad (2)$$

where C is the starch digested (expressed as %db) at incubation time t (min), $1 - C$ is the undigested starch remaining after incubation time t and K is the digestion rate coefficient (min^{-1}).

For the purpose of data fitting, the value of K was obtained by a linear-least-squares fit of the solution of Eq. (2), viz., as the slope of a plot of $\ln(1 - C)$ against t for each granule size. The linearity of such a plot also provides a measure of the applicability of first-order kinetics (Al-Rabadi et al., 2009).

3. Results and discussions

3.1. Effectiveness of sedimentation techniques for separation of starch

Most previous separation procedures for starch granules have been based on either sedimentation in water or sieving (wet or dry) of the granules (Ao & Jane, 2007; Sahal & Jackson, 1996; Takeda et al., 1999; Yamazaki & Wilson, 1964). The sedimentation technique developed in this work, the modified 'Pipet' method, provides an alternative approach with the advantage that granules can be separated into desired size ranges with minimum instrumental setup. Several assumptions were made during the experimental design. It was assumed that the terminal velocity was attained as soon as starch granule settling begun, and that the settling rate was entirely due to the resistance caused by the viscosity of water. Further, starch granules were assumed to be smooth and spherical, without any interaction between individual granules in the solution during separation. As, neither maize nor potato starch are completely spherical and may not fully comply with other assumptions, the size of separated granules is regarded as an apparent size rather than actual size. Similarly, granule size analysis by laser light scattering (Malvern Mastersizer) is also based on the assumption of homogeneity and sphericity of starch granules. Comparison of the predicted size from Stokes law with light scattering measurements of fractions (Table 1) demonstrates the separation efficiency of the proposed method. All the median and surface weighted mean diameters of fractionated MS and PS fell into the predicted size range, showing that the proposed sedimentation technique can be successfully used for separation of maize and potato starches to desired size fractions, based on Stokes law calculations. The 10th and 90th percentile values obtained from the Mastersizer demonstrate the relatively narrow distribution of particle sizes that can be obtained. The general efficacy of this method for other starches depends on their homogeneity and sphericity. For example, the method did not give good separation when applied to high amylose maize starches (Gelose 50 and Gelose 80) (data not shown), as they are much more irregular in shape than MS and PS (Dhital, Shrestha, & Gidley, 2010). The granule diameter can be converted to specific surface area (m^2/g) provided the granule density is constant. Other techniques such as photomicrographic measurements or direct methods like gas or liquid adsorption have been used to determine the specific surface area of starches. The value reported for MS, as determined by nitrogen adsorption, 0.58–0.70 m^2/g (Hellman, Boesch, & Melvin, 1952; Juszczak, Fortuna, & Wodnicka, 2002; Sujka & Jamroz, 2009), is higher than the calculated value from light scattering (Table 1). The surface pores (Fig. 2A, D, G and J), channels and cavities characteristic of maize starch (Fannon, Hauber, & Bemiller, 1992; Fannon, Shull, & Bemiller, 1993; Huber & BeMiller, 1997) form an internal surface that would be expected to be detected by gas adsorption, increasing the specific surface area value compared to that calculated from the granule diameter by light scattering. However, for PS, which seems to lack obvious surface pores (Fig. 3A, D, G, J and M) and channels, the specific surface area determined from nitrogen adsorption, 0.11–0.16 m^2/g (Hellman et al., 1952; Sujka & Jamroz, 2009) is similar to the calculated value from light scattering (Table 1).

3.2. Enzymatic hydrolysis of starches

Irrespective of granule size, both MS and PS exhibited monophasic digestograms (Fig. 4). The longer the substrate–enzyme contact time, the more starch was digested within the duration of the experiment, with almost all MS digested within 8 h. MS was more rapidly digested compared to PS at each time point. This could be due to the known differences in granular, supramolecular and/or molecular structures (Buleon et al., 1998) between these starches. MS and PS exhibit A and B-type crystalline polymorphs respectively (Lopez-Rubio, Flanagan, Gilbert, & Gidley,

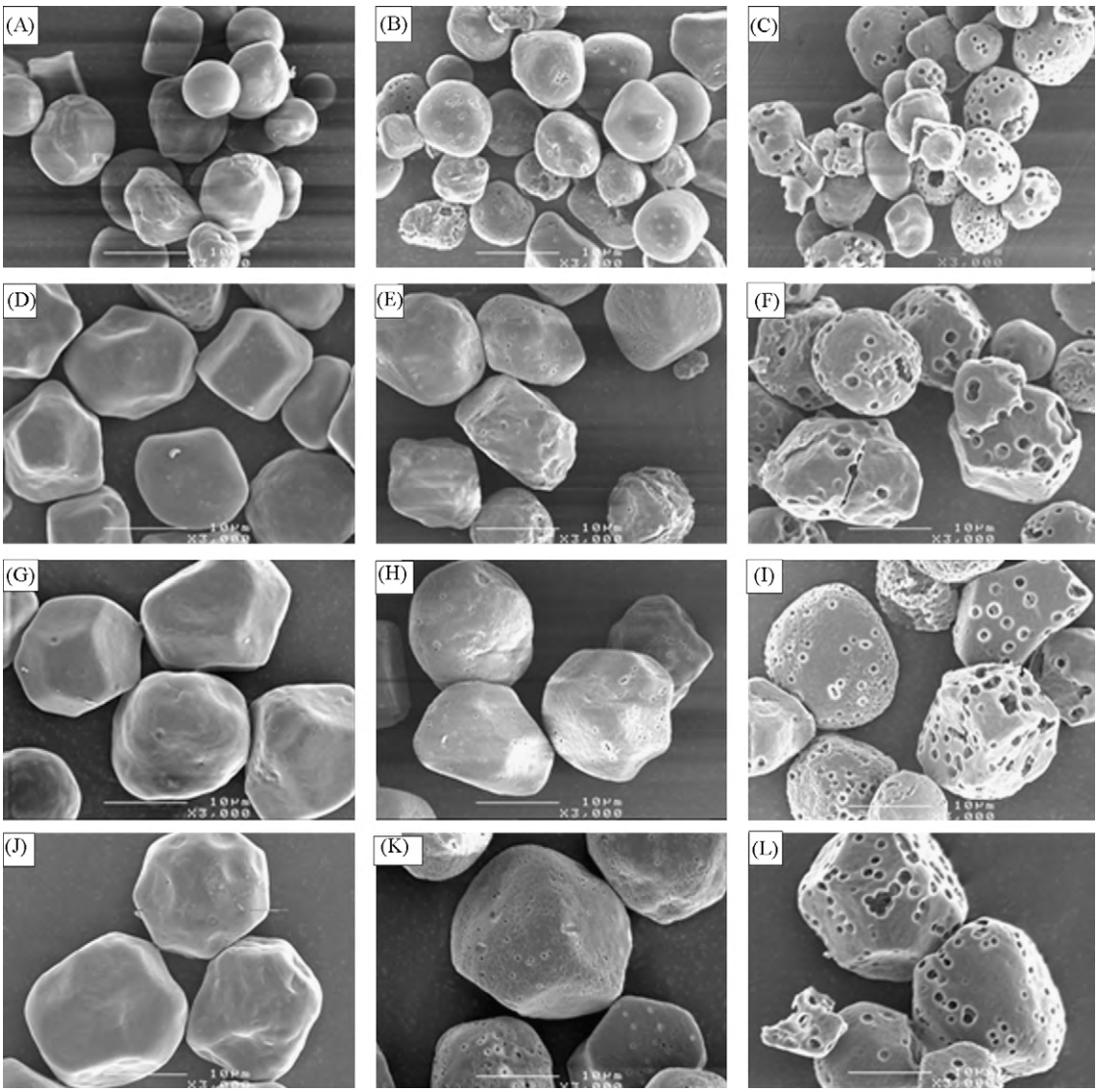

Fig. 2. Scanning electron micrographs of residues from amylase digestion of fractionated maize starch granules: MS-VS control, 20 min and 4 h digestion (A, B and C); MS-S control, 20 min and 4 h digestion (D, E and F); MS-M control, 20 min and 4 h digestion (G, H and I); MS-L control, 20 min and 4 h digestion (J, K and L).

2008; Tan, Flanagan, Halley, Whittaker, & Gidley, 2007). The B-type polymorphs have been proposed to have more branch points in non-crystalline regions, leading to high density amorphous regions and stable crystallites, that resist enzymatic hydrolysis compared to A-type polymorphs (Jane, Wong, & McPherson, 1997). The slow hydrolysis of PS has also been ascribed to larger blocklets of amylopectin lamellae (200–500 nm) compared to MS (20–120 nm) (Gallant, Bouchet, & Baldwin, 1997; Gallant, Bouchet, Buleon, & Perez, 1992; Tang, Mitsunaga, & Kawamura, 2006). A combination of larger granule size and smoother surface, together with specific supramolecular properties have been proposed to provide the resistance of PS against enzymatic digestion (Oates, 1997). On the other hand, smaller granule size (compared to PS), a rough surface and the presence of surface pores and channels (Dhital et al., 2010; Fannon et al., 1992; Fannon et al., 1993) dramatically increases the number of sites for enzyme diffusion and adsorption (Oates, 1997; Zhang, Ao, & Hamaker, 2006) leading to the rapid hydrolysis of MS.

Enzymatic hydrolysis of a native starch is a solid-solution two phase reaction in which the enzyme needs first to diffuse to and adsorb on the solid substrate before catalysing the cleavage of glycosidic linkages (Zhang et al., 2006). Diffusion of enzymes onto the starch surface and then inside the granules, therefore, may be rate-limiting steps in enzymatic hydrolysis. Smaller granules, by virtue of their larger specific surface area (Table 1), facilitate adsorption of enzymes (Colonna et al., 1992) and thus are hydrolysed more rapidly compared to larger granules (Fig. 5). The digestion rate coefficient of smaller fractions of MS and PS were higher than those of larger granules (Table 2) signifying a positive relation between the

Table 2
Digestion rate coefficient (K, min^{-1}) of fractionated maize starch (MS) and potato starch (PS).

MS	K_{MS}	PS	K_{PS}
MS-VS	$(4.79 \pm 0.01) \times 10^{-3}$	PS-VS	$(1.80 \pm 0.02) \times 10^{-4}$
MS-S	$(4.28 \pm 0.03) \times 10^{-3}$	PS-S	$(1.38 \pm 0.03) \times 10^{-4}$
MS-M	$(3.99 \pm 0.04) \times 10^{-3}$	PS-M	$(1.19 \pm 0.03) \times 10^{-4}$
MS-L	$(3.54 \pm 0.01) \times 10^{-3}$	PS-L	$(1.03 \pm 0.03) \times 10^{-4}$
		PS-VL	$(8.88 \pm 0.05) \times 10^{-5}$

K values are significantly different at $P < 0.05$ within each group (GenStat software, release 5 (3.2), VSN International, Hemel Hempstead, UK). Values are means ± standard deviation of two replicates.

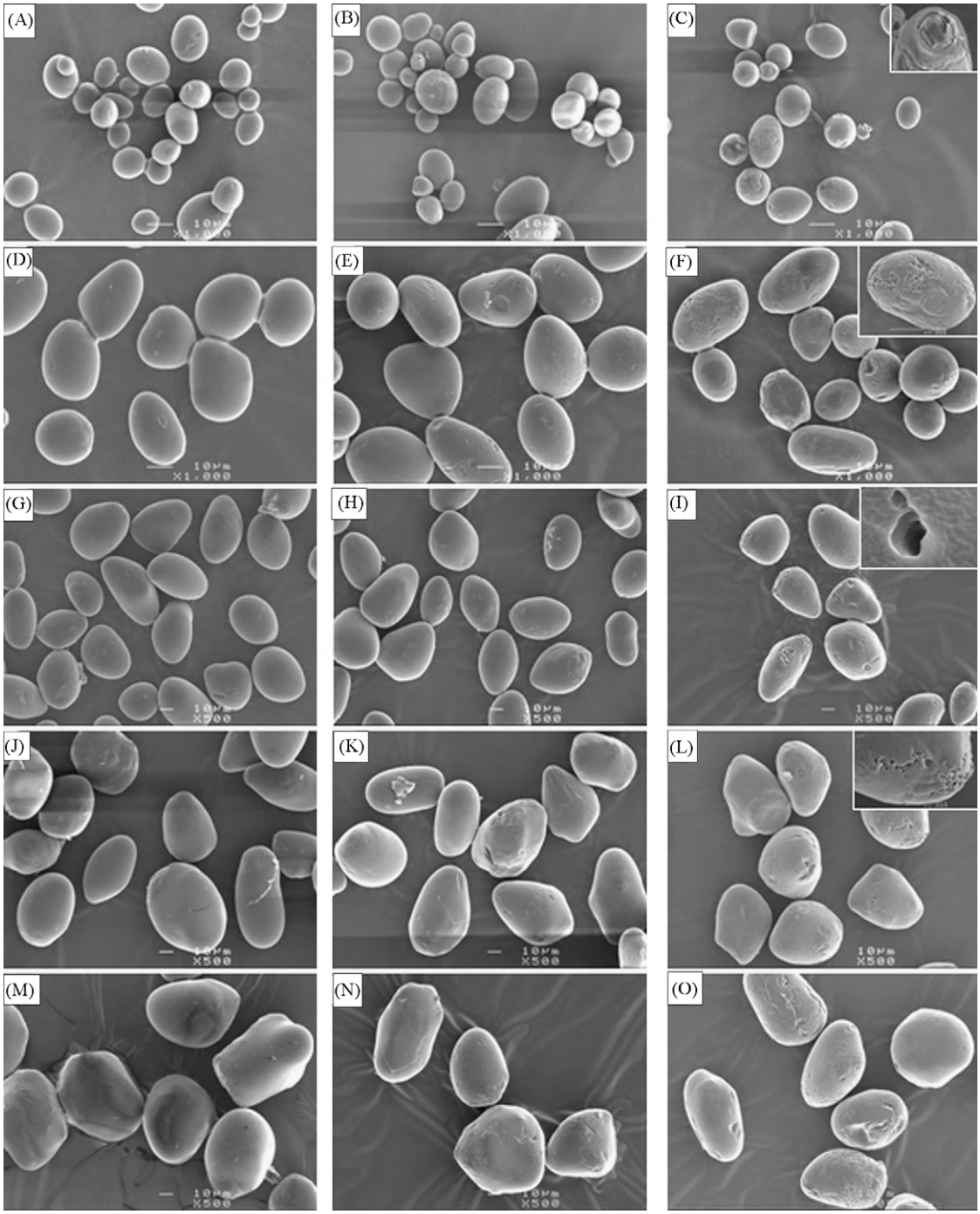

Fig. 3. Scanning electron micrographs of residues from enzyme digestion of fractionated potato starch granules: PS-VS control, 20 min and 24 h digestion (A, B and C); PS-S control, 20 min and 24 h digestion (D, E and F); PS-M control, 20 min and 24 h digestion (G, H and I); PS-L control, 20 min and 24 h digestion (J, K and L); PS-VL control, 20 min and 24 h digestion (M, N and O). Note that magnification is higher for PS-VS and S than for other sizes.

available surface area and hydrolysis rate. The calculated surface area for fractionated MS, however, would be expected to be lower than the actual surface area available to an object of the size of α-amylase (ca. 3 nm radius; Payan et al., 1980) due to the presence of structural features like pores (Fig. 2A, D, G and J), channels and cavities (Fannon et al., 1992; Fannon et al., 1993; Huber & BeMiller, 1997). Correlations of digestion rate coefficient (K) with specific surface area for MS and PS are shown in Fig. 6. PS showed a stronger correlation compared to MS with $R^2 > 0.97$. This may be due to the rougher surface structure of MS where the actual surface area as experienced by the enzyme may deviate more from the calculated value than for PS.

In addition to differences in the size dependence of rate coefficients, there are major differences in digestion rate for similar size fractions of MS and PS (Table 2). M-MS and VS-PS fractions have similar granule sizes with [$D(3, 2)$] averages of 16.6 and 16.0 μm respectively (Table 1) but rate coefficients of $(3.99 \pm 0.04) \times 10^{-3}$ and $(1.80 \pm 0.02) \times 10^{-4}$ min^{-1} respectively. The VS-PS rate coefficient is considerably lower than that of the largest maize fraction which has a [$D(3, 2)$] of 20.2 μm and a rate coefficient of

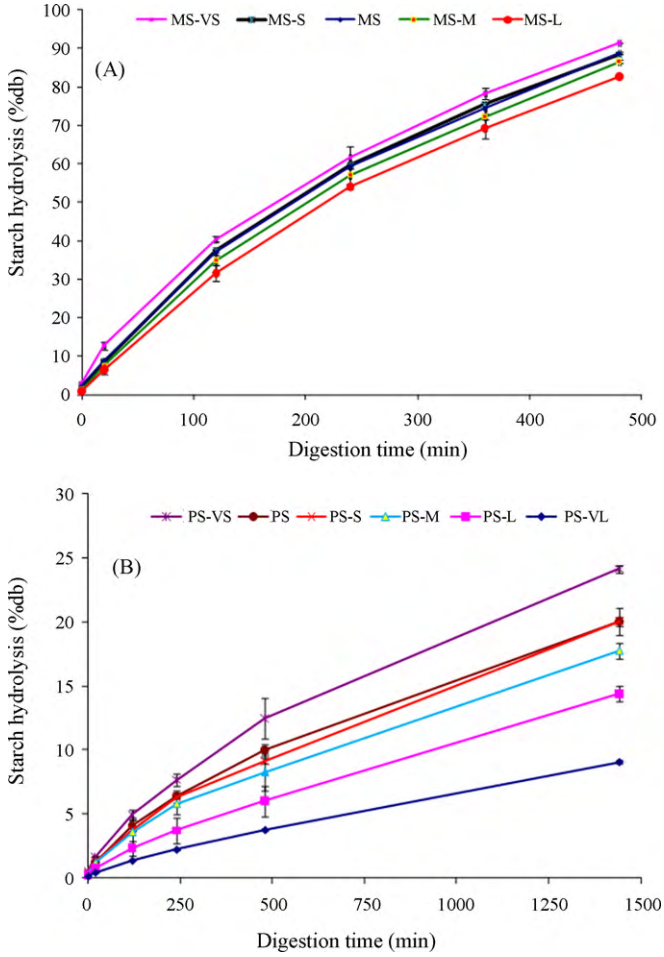

Fig. 4. Digestograms of fractionated starches: MS (A) and PS (B).

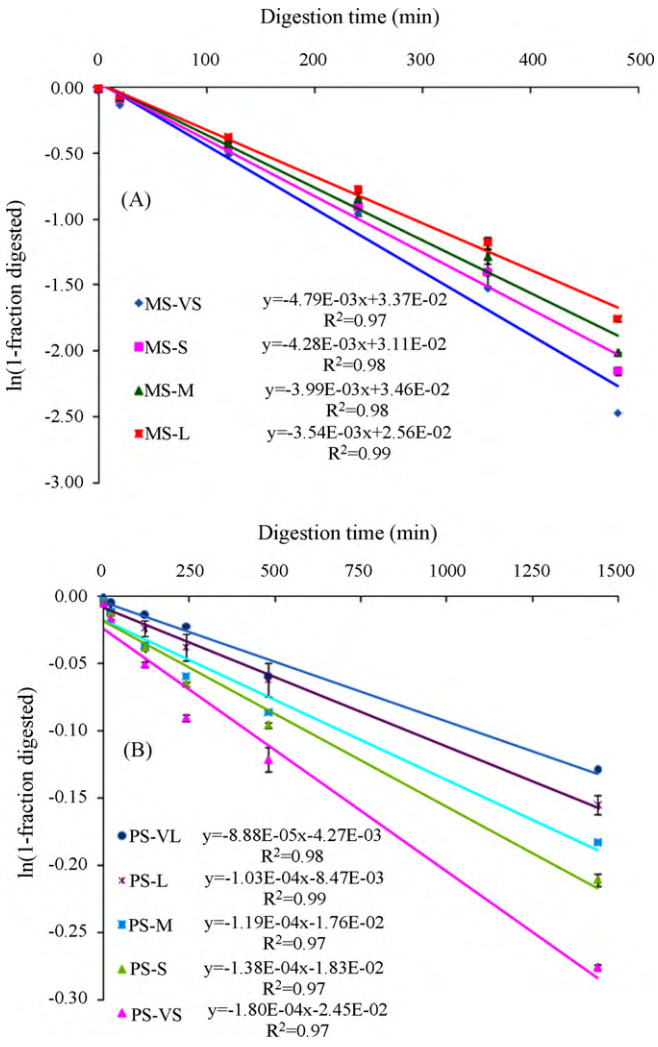

Fig. 5. Fit of first-order kinetics to digestograms of fractionated starch granules. The slope (digestion rate coefficient (K)), intercept and coefficient of determination (R^2) are shown for each separated fraction of MS (A) and PS (B).

$(3.54 \pm 0.04) \times 10^{-3}\ \text{min}^{-1}$. It is clear that there is a quantitative difference between MS and PS that is much more important than granule size in determining relative enzyme digestion rates.

3.3. Diffusion model of enzymatic hydrolysis of fractionated starches

Assuming spherical particles, the surface area is directly proportional to the square of the radius or diameter of the particle. Moreover, if the rate-determining step is assumed to be the diffusion of enzyme onto the surfaces of spherical particles, the rate of digestion from first-order kinetics is related to the average particle size by

$$\frac{1}{K} = \left(\frac{X^2}{6D}\right) \tag{3}$$

where K, X and D represents digestion rate coefficient (s^{-1}), starch granule size (cm) and diffusivity coefficient ($cm^2\ s^{-1}$) respectively. Thus, if surface adsorption is rate-limiting, Eq. (3) implies that a plot of the reciprocal of K against the square of the particle size should be linear. This is indeed what is found for both MS and PS (Fig. 7). How-

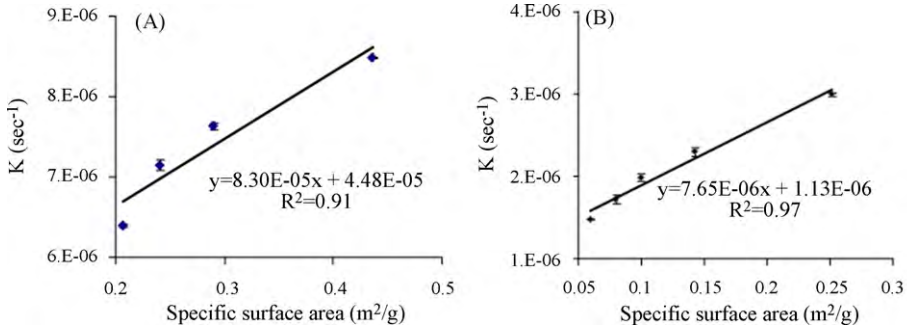

Fig. 6. Fit of digestion rate coefficient (K) as a function of specific surface area for MS (A) and PS (B).

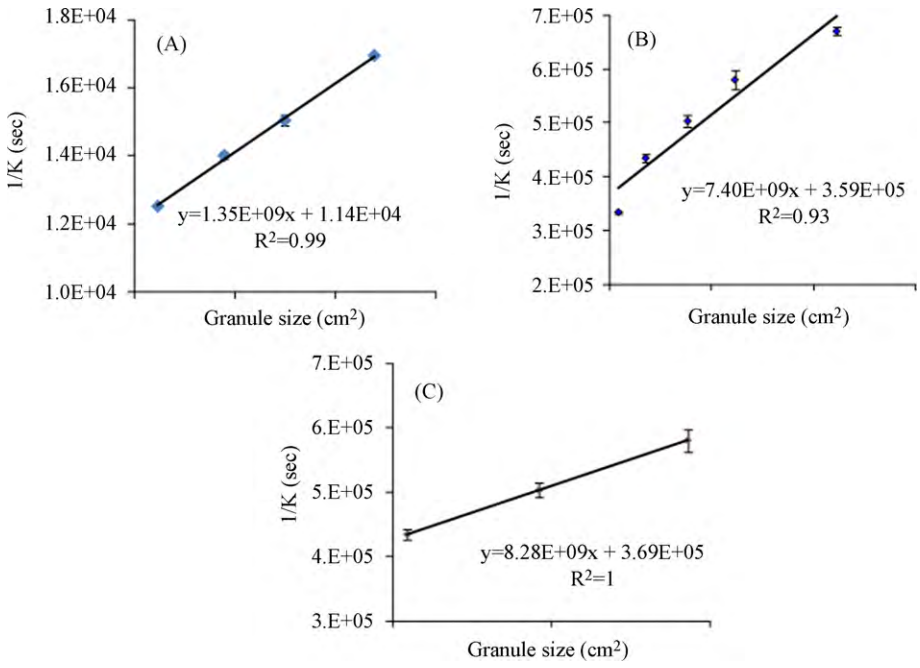

Fig. 7. Fit of reciprocal of digestion rate coefficient (K) as a function of average granule size squared for MS (A), PS (B) and PS excluding the two extreme data points(C).

ever, rate-limiting surface adsorption is not a unique explanation of this behaviour as it may be the diffusion of enzyme to the granule surface that is rate-determining (i.e. D is the main determinant rather than X). This mechanism has been invoked to account for the size dependence of rate coefficients of amylase digestibility for milled barley and sorghum grain fractions (Al-Rabadi et al., 2009; Mahasukhonthachat et al., 2010). Since the experiments described here used pure granular starches, we postulate that the barrier in this system is only the effective granule surface as sensed by the enzyme (not necessarily determined only by granule diameter). This contrasts with grain particles, where the rate-limiting step is considered to be movement of the enzymes within the particles, with protein matrices and cell walls acting as barriers controlling enzyme diffusion to starch granule surfaces, with subsequent hydrolysis being faster than the diffusion of enzymes to the starch (Al-Rabadi et al., 2009).

The relationships between digestion rate coefficient (K) and granular size of fractionated MS and PS (Fig. 7A and B) are given by Eqs. (4) and (5) respectively:

$$\frac{1}{K_{MS}} = 1.35E{+}09(X_{MS})^2 + 1.14E{+}04 \quad (4)$$

$$\frac{1}{K_{PS}} = 7.40E{+}09(X_{PS})^2 + 3.59E{+}05 \quad (5)$$

where K_{MS} and K_{PS} are digestion rate coefficients for maize starch and potato starch respectively (Table 2) and X_{MS} and X_{PS} are the square of $D(3, 2)$ values (cm^2) obtained from Table 1. The apparent diffusion coefficient obtained using these fits and Eq. (3) are $D = 7.40 \times 10^{-10}$ and $1.35 \times 10^{-10}\ cm^2\ s^{-1}$ for MS and PS respectively. A better fit is obtained for PS (Fig. 7C) when the two extreme data points (PS-VS and PS-VL) which represent all granules smaller than or larger than a cut-off value are excluded. The diffusivity coefficient (D) calculated from Eq. (6) is $1.20 \times 10^{-10}\ cm^2\ s^{-1}$, with a R^2 value of unity.

$$\frac{1}{K_{PS}} = 8.28E{+}09(X_{PS})^2 + 3.69E{+}05 \quad (6)$$

Similar *in vitro* digestion experiments conducted for milled barley and sorghum grain particles showed diffusivity coefficients to be in the order of $10^{-7}\ cm^2\ s^{-1}$ (Al-Rabadi et al., 2009; Mahasukhonthachat et al., 2010). This represents a difference of the order of 10^3 compared with the present data, and is consistent with starch granule hydrolysis being faster than enzyme diffusion through a grain matrix.

If enzyme diffusion to the granule surface is also rate-limiting for isolated granules, the present results imply that under the conditions of the experiment, amylases diffused more than 5 times faster in the maize system compared to the potato system. This is physically unreasonable, as starch granules were freely available to enzymes in an aqueous dispersion. We therefore propose that the effective granule surface area is the rate-limiting factor. As the rate coefficients and their granule size dependence are so different for maize and potato starches (Table 2), light scattering or microscopy measurements which report on the external dimensions of granules do not apparently reflect the effective surface area available for enzyme attack. It is therefore likely that the pores (Fig. 2A, D, G and J), channels and cavities, characteristic of maize starch (Fannon et al., 1992; Fannon et al., 1993; Huber & BeMiller, 1997) and not potato starch (Fig. 3A, D, G, J and M), cause maize starch to have a much higher effective surface area and therefore digestion rate compared to potato starch. Qualitative support for this is provided by electron micrographs of partially digested granules (Figs. 2 and 3). In common with previous descriptions, maize starch granules seem to be digested through numerous pores, whereas potato starch granules apparently have a surface 'barrier' which, once breached allows access of enzyme to the interior for all granule sizes (Figs. 2 and 3). Diffusion of amylase and the consequent catalytic patterns in maize and potato starches are presented schematically in Fig. 8. Thus the present results are consistent with previous observations and for the first time provide quantitative parameters that define the major difference in enzyme susceptibility of the maize and potato starch granules. It is still unclear how pores and channels are formed in certain starch granules. Jane et al. (2003) isolated starch granules from different parts of the corn kernel. They reported that starch granules near to the germ contained more pores than granules from other parts of the kernel, and suggested that this was a consequence of active *in situ* amylase(s). However, channels (pores) of A polymorphic starches

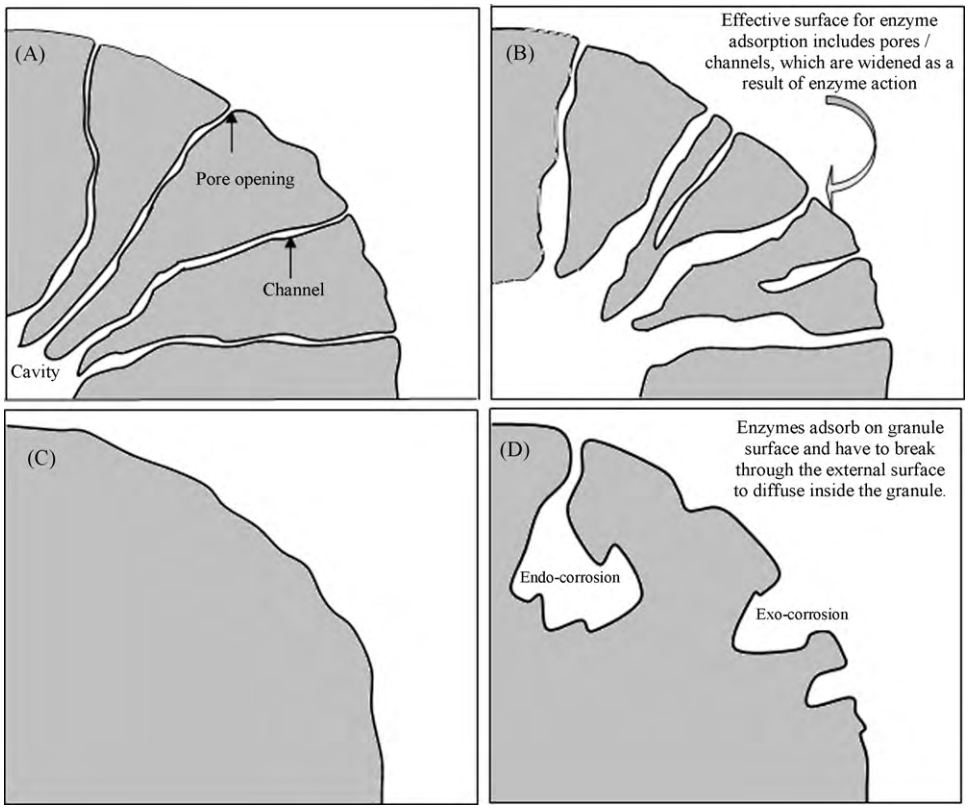

Fig. 8. Model illustrating diffusion of amylase and its catalytic patterns in maize and potato starches: maize starch showing pores, channels and cavity (A), maize starch hydrolysed by amylase with enlarged pores, channels and cavity (B), potato starch lacking pores, channels and cavity (C), and potato starch exo- and endo-corroded by amylase (D).

contain proteins similar to those found in the amyloplast, suggesting that channels (pores) are formed as granules develop around radially oriented microtubules in the amyloplast (Fannon, Gray, Gunawan, Huber, & Bemiller, 2004; Han & Hamaker, 2002).

Assuming that potato starch hydrolysis is determined by external surface area, a calculation can be made based on trend line equation from Fig. 6B ($K = 7.65E{-}06X + 1.13E{-}06$) to predict the size of potato starch granule that would have the same digestion rate coefficient as maize starch (here taken as $7.15E{-}05\ s^{-1}$). The predicted surface area is found to be $9.19\ m^2/g$, and the equivalent diameter of a sphere of density $1500\ kg/m^3$ is calculated to be 0.435 μm. Although these calculations assume a similarity between maize and potato starch that is difficult to defend, the predicted diameter is of a similar size range as the distance between detectable pores/channels in maize starch (Fig. 2). This leads us to suggest that the dominant reason for the large difference in enzyme hydrolysis between potato and maize starches is the presence of enzyme-accessible channels in maize starch, rather than e.g. crystallite polymorph or amylopectin blocklet size differences (Buleon et al., 1998; Gallant et al., 1997; Gallant et al., 1992; Tang et al., 2006).

One limitation of these studies is that the fractionated granules are assumed to be similar in terms of their structures at different length scales. It is possible that structural (molecular and supramolecular) difference could also affect enzymatic hydrolysis rates. In order to address this, the molecular and supramolecular structure of fractionated granules is under study and will be reported subsequently.

4. Conclusions

Starch granules such as those from maize and potato with regular shapes and apparent unimodal size distributions can be separated into pre-determined size fractions by use of a simple instrumental setup (Fig. 1) based on Stokes law of sedimentation. The *in vitro* digestibility of fractionated maize and potato starch granules are well fitted by an inverse square relationship between digestibility coefficient and granule size, consistent with a surface area-controlled mechanism. Structural features like surface pores and channels are proposed to increase effective surface area, and facilitate the rapid diffusion of amylases to substrates in maize starch compared to potato starch lacking such structural features.

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