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The Effect of Granule Size on the Digestibility of Wheat Starch using an *in vitro* Model

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***Abstract*—**Wheat has a bimodal starch granule population and the dependency of the rate of enzymatic hydrolysis on particle size has been investigated. Ungelatinised wheaten starch granules were separated into two populations by sedimentation and decantation. Particle size was analysed by laser diffraction and morphological characteristics were viewed using SEM. The sedimentation technique though lengthy, gave satisfactory separation of the granules. Samples (<10µm, >10µm and original) were digested with α-amylase using a dialysis model. Granules of <10µm showed significantly higher rate of reducing sugar release than those >10µm (p<0.05). In contrast, the rate was not significantly different between the original sample and granules >10µm. Moreover, the digestion rate was dependent on particle size whereby smaller granules produced higher rate of release. The methodology and results reported here can be used as a basis for further evaluations designed to delay the release of glucose during the digestion of native starches.

Keywords*—in vitro* Digestion, α-amylase, wheat starch, granule size.

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I. Introduction

DIGESTION involves the chemical breakdown of food into molecules that are sufficiently small to facilitate absorption into the blood stream [1]. In humans, digestion starts from the mouth and ends at the anus.

Unlike micronutrients (vitamins and minerals), macronutrients including carbohydrates, are broken down in a series of steps along the gastrointestinal tract with up to 95% of absorption occurring in the small intestine [1]. Carbohydrate digestion is initiated from the mouth where food is broken down into smaller pieces through mastication and mixed with salivary secretion. This moistens the food and among other components contains α-amylase that starts the breakdown of starch into smaller fragments [2]. Salivary α-amylase is inactivated by the acidic environment of the stomach where digestion is primarily focused on proteins and fat [2]. The porridge-like food mixture, chyme, from the stomach is gradually released into the small intestine that can measure up to 3m in length with diameter of 25-30mm in humans [3]. There, pancreatic α-amylase continues the hydrolysis of starch structures, involving depolymerisation and the products are dextrins and oligosaccharides. Pancreatic α-amylase has similar catalytic actions as salivary α-amylase having multiple attack mechanisms [4] despite having a different amino acid sequence, hence if adequate amounts of pancreatic α-amylase are secreted, digestion of starch can be completed independently of the action of salivary α-amylase [3].

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Dextrinase and glucoamylase thereon convert maltose into glucose. Intestinal juices contain a range of other digestive enzymes including maltase, lactase and sucrase which hydrolyse the disaccharides maltose, lactose and sucrose into their respective monosaccharides.

The purpose of this work has been to compare the effect of granule size on the digestibility of native wheat starch. As most of starch digestion occurs in the small intestines, a dialysis approach has been applied to mimic the digestive system and providing a means of controlling viscosity of the samples tested [5].

II. Materials and Methods

A. Materials

Ungelatinised wheaten corn flour starch was obtained from Starch Australasia Ltd. Porcine pancreatin was sourced from Megazyme (E-PANAA). Cellulose dialysis membranes (453105) with molecular weight cut-off (MWCO) of 12kDa, having flat width (FW) of 42mm, were obtained from Science Supply, Australia.

B. Particle separation by sedimentation

Wheaten starch granules were separated in two populations: <10µm and >10µm by an adapted sedimentation technique [6]. Starch (20g) was made up to 1L with deionised water in a 1L measuring cylinder. Using a hand stirrer, the starch was dispersed with lateral movement based on Stokes law for 30s. The mixture was left undisturbed for 61min and the top 10cm of the mixture was decanted using a 10mL pipette connected to a vacuum inlet and an adjustable Drechsel head. The remaining mixture was filled up to 1L again and the procedure repeated 13-15 more times until the top layer was clear. The decanted mixture was left to settle overnight until all starch was deposited. Water was decanted and the remaining mixture frozen with liquid nitrogen and freeze dried overnight. Triplicate preparations were made.

C. Particle size analysis

Samples were dispersed in Milli-Q water in a flowing cell and particle size distribution measured using the Malvern Mastersizer X equipped with a 45mm lens.

D. Surface morphology

Samples were visually evaluated for size and morphological changes with the Environmental Scanning Electron Microscope (ESEM). Dried samples were mounted on copper stubs with double-sided carbon tape, coated with a gold sputter coater unit and viewed under low vacuum by a FEI Quanta 200 ESEM with accelerating voltage of 25-30kV and spot size 4.0.

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E. in vitro Digestion

A dialysis tubing model was adapted [7] and analyses duplicated. A beaker of 800mL of 0.02M phosphate buffer containing 0.02M $CaCl_2$ and 0.03M NaCl at pH 6.9 and 37ºC was prepared. Starch sample (2g) from wheat was suspended in 15cm of dialysis membrane with 15mL of buffer and 5000U α-amylase. This was suspended into the preequilibrated beaker and digested with gentle stirring for three h. Aliquots (0.5mL) from the dialysed solution were taken immediately for time 0 and thereafter every 30min for.

F. Determination of rate of reducing sugar release by DNS reagent method

Dialysed aliquots were mixed with dinitrosalicylic acid (DNS) reagent and heated at 100ºC for 15min [8], [9]. A_{540} was recorded with a Cary 50 Bio UV-visible spectrophotometer. A glucose standard curve was plotted for each analysis (not shown).

G.Cross sectional analysis

Following digestion, subsamples of wheat starch granules were frozen with liquid nitrogen and sliced with a metal scalpel. They were mounted on copper studs, gold coated and viewed by the ESEM as described above.

H.Moisture content

Moisture content of wheaten starch and sedimented samples (2g) were analysed using an air oven preequilibrated at 130 ± 3ºC [10]. Subsamples were dried to constant weight and moisture content was calculated using the following equation.

$$\text{Moisture content (\%)} = \frac{\text{Loss in weight of sample upon drying (g)}}{\text{Initial weight of sample (g)}} \times 100$$

I. Statistical analysis

One way ANOVA with posthoc tests at 95% confidence interval were performed using SPSS Statistics 17.0 software.

III. Results and Discussion

A bimodal population from the original wheat starch sample was confirmed with two clearly differentiated populations, starch granules <10µm and >10µm in diameter (Fig. 1 and 2). From the sedimentation technique, a recovery of 92.6 ± 0.8% starch (expressed on a dry weight basis) was obtained. The two primary populations (<10µm and >10µm, Table 1) as well as the original unseparated wheat sample were digested and the data for release of reducing sugars was plotted (Fig. 3).

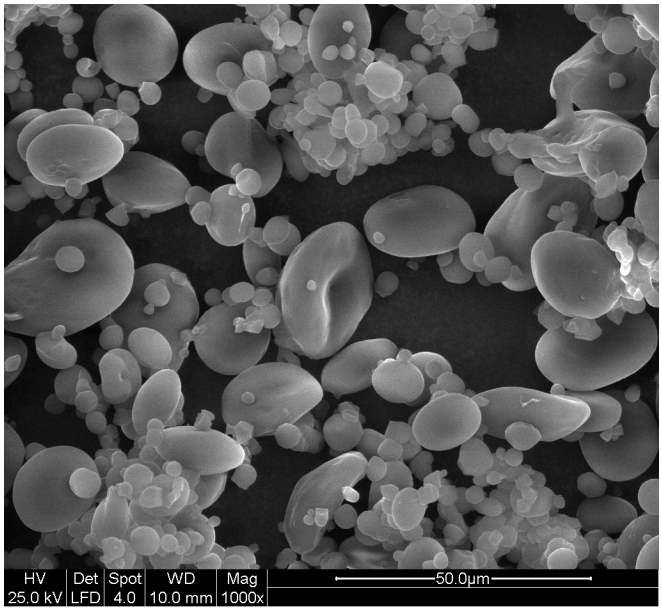


Fig. 1 Starch recovered inside dialysis tubing without α-amylase

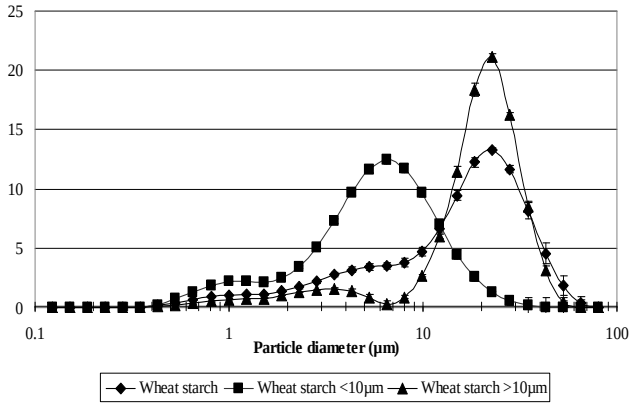


Fig. 2 Particle size distribution of starch fractions after sedimentation

TABLE I
COMPOSITION OF WHEAT STARCH BASED ON PARTICLE SIZE

Granule diameter	% weight* ± RSD
<10µm	29.2 ± 1.6
>10µm	70.8 ± 0.7

* dry weight basis.
RSD: relative standard deviation

Wheat starch granules of <10µm showed significantly higher rates of reducing sugar release than the granules >10µm (p<0.05). This confirms previous observations that the smaller the particle size, the greater is the surface area of substrate being exposed to enzymatic attack and hence the higher the rate of digestion. As the small and larger fractions of starch granules were subjected to similar treatment conditions during the separation process, it is likely that a direct comparison of rates can be made. The results obtained in the current do not preclude the possibility that the surfaces of some of the starch granules may have included a layer of resistant starch [11], [12].

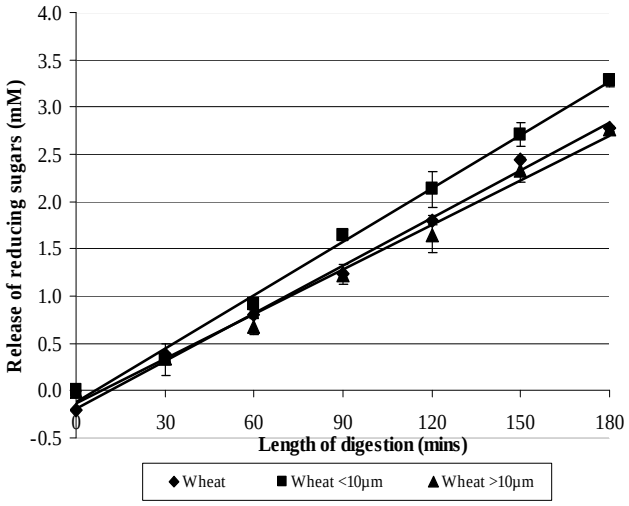


Fig. 3 Rate of reducing sugars released from *in vitro* digestion

The rate of reducing sugar release of the original starch sample was not significantly different from that of either of the wheat starch fractions (<10µm and >10µm, $p<0.05$). Less than one third of the starch granules were <10µm in the original starch sample thereby resulting in these having a minimal effect on the overall rate of release of reducing sugars during digestion (Table 1).

Morphological changes upon *in vitro* digestion showed a change from an initial smooth surface on the undigested starch granules (Fig. 1) to patterns of exocorrosion on digested samples. Typically, similar surface pitting in conjunction with internal channels giving rise to a sponge-like appearance were observed from the original wheat starch and with the granules >10µm in size (Fig. 4 (a) and Fig. 4 (b) respectively). These observations are similar to those described by other researchers [12], [13], [14].

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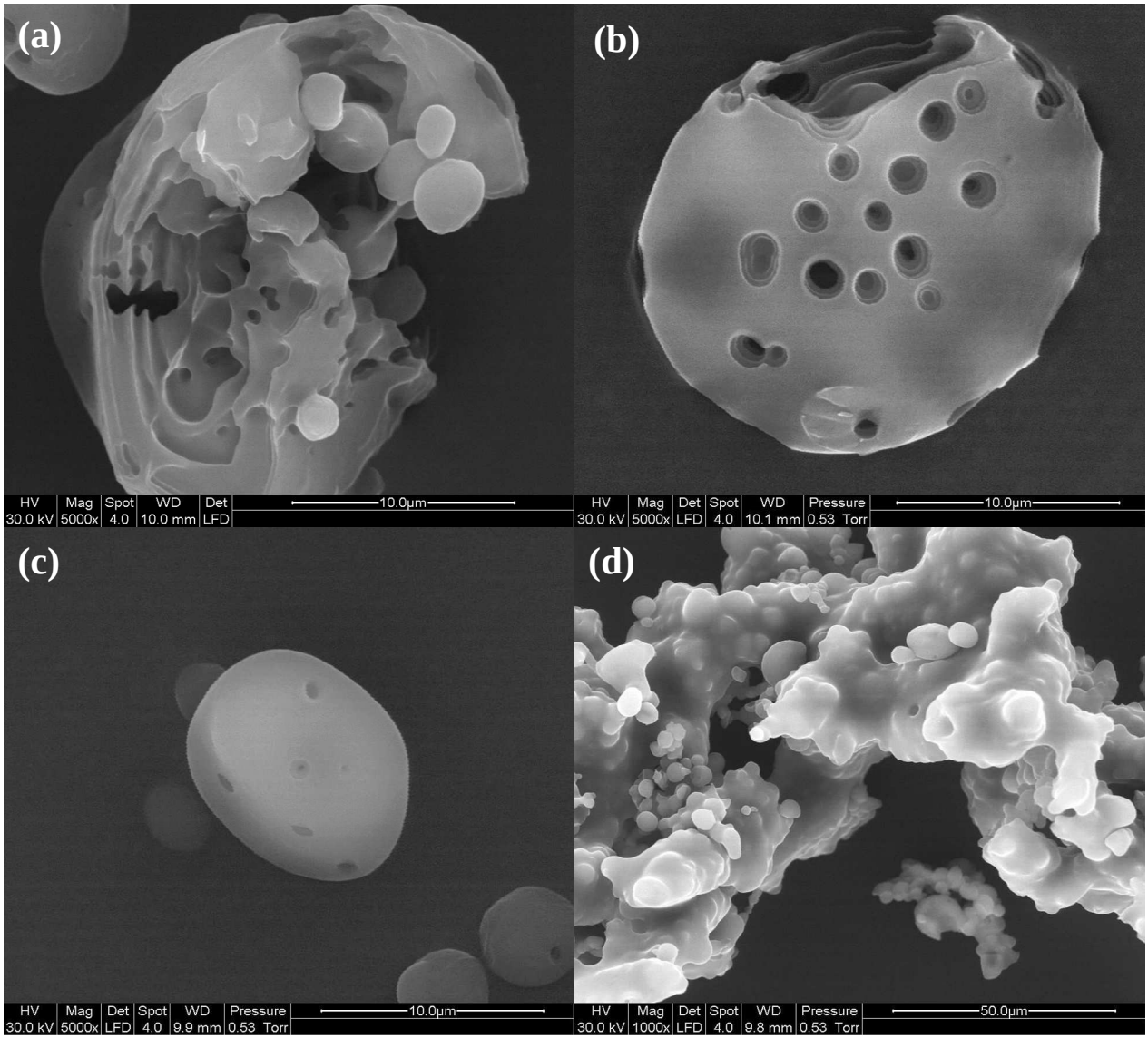


Fig. 4 ESEM images post digestion at magnification 5000×(a) = original wheat starch, (b) = granules >10µm, (c) = granules <10µm, (d) = granules <10µm at magnification 1000×

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α-Amylase appears to favour attack at the outer crease of the starch granule rather than at other sites on the surface. In the original wheat sample, uneven amylolysis within the distribution of starch granules was observed with the larger granules of the bimodal wheat starch distribution being more obviously subjected to hydrolytic action. This may indicate that smaller granules are more resistant to hydrolysis than larger granules based on observations of the ESEM images as discussed by other researches [12], [15], [16]. However, when <10μm digested granules were viewed in the ESEM, similar pitting were found even though damaged starch granules appear to be more sparsely distributed (Fig. 4 (c)). Moreover, in one of the images of digested granules <10μm, there appears to be a relatively large amount of amorphous material from the partial breakdown of granular starch and which was not readily dialyzable (Fig. 4 (d)). This is consistent with a greater release of molecular starch during digestion as well as a higher digestion rate than the other samples and hence confirms the higher rate of reducing sugar release seen from Fig. 3.

Digested starch granules primarily showed extensive pitting with a limited number of granules demonstrating exochanneling (Fig. 5). However, from cross-sections of the starch granules, there is clear evidence that the pitting led to extensive internal channeling (Fig 6) confirming observations by other researchers [13], [14]. This might ultimately result in the starch granule becoming weakened to the extent that collapse ensues. Moreover, the appearance of the cross-sections also highlights the presence of the characteristic growth rings within the wheat starch granule and the preferential erosion of the material between the growth rings [16], [17].

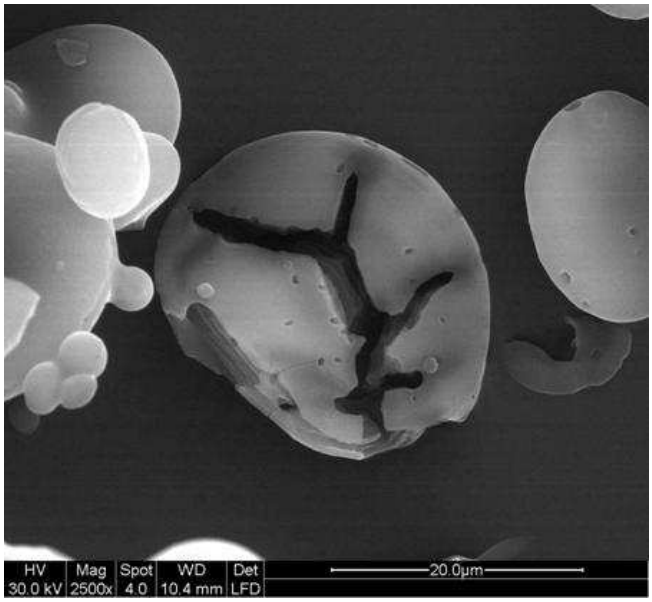


Fig. 5 Surface channel formation post digestion

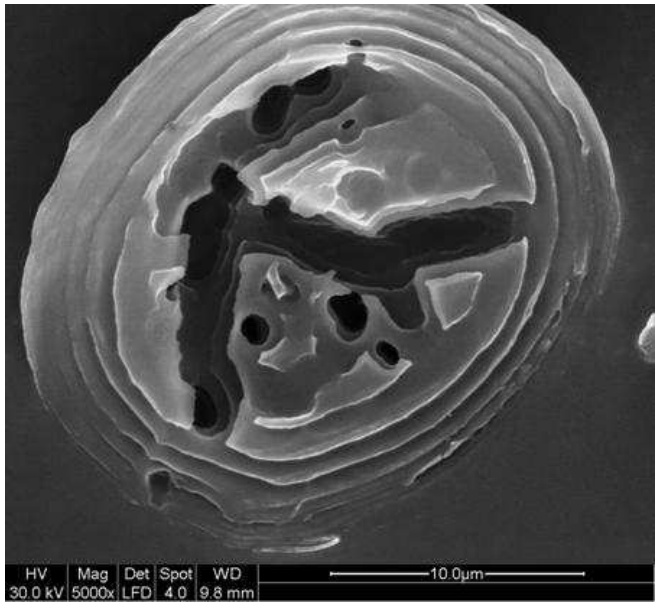


Fig. 6 Cross section of digested starch granule

IV. Conclusion

The sedimentation technique, although lengthy, gave satisfactory separation based on particle size. This study showed that native wheat starch of smaller particle size gave significantly higher rates of release of reducing sugar upon digestion. Moreover, both small and larger starch granules appear to undergo similar morphological changes during enzymatic attack.

Acknowledgment

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