Advance Journal of Food Science and Technology 12(4): 201-208, 2016
DOI:10.19026/ajfst.12.2899
ISSN: 2042-4868; e-ISSN: 2042-4876
© 2016 Maxwell Scientific Publication Corp.
Submitted: September 22, 2015 Accepted: October 30, 2015 Published: October 05, 2016

Research Article

Comparative Study on the Effect of Granule Size (Similar Composition) of Wheat on the Extent of Granular Starch Hydrolysis

U. Uthumporn, N.Y. Shariffa and A.A. Karim
Food Technology Division, School of Industrial Technology, Universiti Sains Malaysia, 11800, Minden, Penang, Malaysia

Abstract: The effect of granule size on the susceptibility of wheat starch towards granular starch hydrolysis (35°C) was investigated. Big and small granules size of wheat was hydrolyzed in granular state by using granular starch hydrolyzing enzyme for 24 h. Hydrolyzed small wheat granules showed the highest percentage of DE compared to big wheat granules with 37 and 27%, respectively. SEM micrographs showed the presence of porous granules and surface erosion in both small and big wheat starch granules compared to their control counterparts. X-ray analysis showed no changes but with sharper peaks for all the hydrolyzed starches, suggested that hydrolysis occurred on the amorphous region. The amylose content and mean diameter of wheat starch was markedly altered after hydrolysis. Evidently, this enzyme was able to hydrolyze granular starches and different granule size significantly affected the degree of hydrolysis with small wheat granules showed more susceptibility to granular starch hydrolysis.

Keywords: Enzymes and dextrose equivalent, granular starch hydrolysis, starch

INTRODUCTION

Starch is the most abundant form of storage polysaccharides in higher plants. In starch granules, amylose and amylopectin are densely packed in a semi crystalline state with inter- and intra-molecular bonds, they are insoluble in cold water and are often resistant to chemicals and enzymes. Starch from any source can be used as an inexpensive source for the production of fermentable sugars containing glucose, fructose or maltose, all of which are widely used in food industries. In addition, these sugars can be fermented to produce bio-ethanol.

Previous investigations of granule size distribution of wheat starch have reported a bimodal distribution (Li *et al*., 2008). Mature wheat endosperm contain two types of starch granules: large (10-35 μm) A- type and small (1-10 μm) B type. There is, however, no clear separation and granules of intermediate sizes also exist (Lindeboom *et al*., 2004). Big-type starch granules contribute to >70% of the total weight and ~3-5% of the total granules of endosperm starch, whereas small-type starch granules account for >90% of the total granule number but <30% of the total weight of starch in wheat endosperm (Raeker *et al*., 1998). The amylose content of wheat starch range between 24-30% depending on the wheat variety and the difference between the two populations of granules is small (Manelius *et al*., 1997).

In the course of conventional enzymatic liquefaction, slurry containing 15-35% starch is gelatinized, where it is heated to 105°C to physically disrupt the granule and open the crystalline structure for the enzyme action (Singh and Soni, 2001). This increases the viscosity of the slurry by 20-fold (Robertson *et al*., 2006) and therefore makes mixing and pumping difficult. The gelatinized starch is liquefied with thermostable alpha-amylase and is then saccharificated with glucoamylase at a much lower temperature of 50-60°C. The whole process requires a high-energy input, which increases the production cost of inverted sugar products. In view of energy costs, effective utilization of natural resources and viscosity (handling) problems, direct hydrolysis of starch below gelatinization temperature is desirable. In recent years, the importance of the enzymatic liquefaction of raw starch without heating has been well recognized, mainly due to energy savings and the effective utilization of biomass, which reduces the overall cost of starch processing (Robertson *et al*., 2006). This has generated a worldwide interest in the discovery of amylases that are capable of digesting raw starches and that do not require gelatinization. The findings of this research are significant to understand the potential of utilizing the granular starch hydrolyzing enzyme for low energy hydrolysis of different starches for the production of fermentable sugars or bioethanol.

Corresponding Author: U. Uthumporn, Food Technology Division, School of Industrial Technology, University Sains Malaysia, 11800, Minden, Penang, Malaysia, Tel.: +604-6532220; Fax: +604-6573678
This work is licensed under a Creative Commons Attribution 4.0 International License (URL: http://creativecommons.org/licenses/by/4.0/).

The previous findings suggest that granule size distribution of wheat starch is an important characteristic that can influence chemical and physical properties. Several studies have demonstrated that starch is digested at varying rates (Slaughter *et al*., 2001). Factors due to processing of the grains will naturally influence the starch digestibility, but starch structure itself is also considered to have an impact on the starch degradation (Topping *et al*., 1997; Weurding *et al*., 2001). Earlier studies have shown that the hydrolysis rate for waxy, normal and high-amylose maize starches was proportional to the surface area of granules, which may be closely related to the adsorption of enzyme onto the granule surface (Li *et al*., 2004). These factors may also play a role regarding the rate and extent of starch hydrolysis. Thus, in this research, wheat starch was fractionated using sedimentation method to regain two types of wheat granules; large (A) and small (B) granules before being hydrolyzed in granular state using Granular Starch Hydrolyzing Enzyme (GSHE). The objective of the experiment reported was to study starch composition and granular structure of wheat and to reveal the relationships between granule size (small- and large-granule of wheat starch) and granular starch hydrolysis.

MATERIALS AND METHODS

Materials: Wheat starch was obtained from SIM Company Sdn. Bhd. (Penang, Malaysia).

The granular starch hydrolyzing enzyme (liquid form), STARGEN 001 enzyme is a product of Genencor International (Palo Alto, CA). STARGEN 001 enzyme contains *Aspergillus kawachi* α-amylase expressed in *Trichoderma reesei* and a glucoamylase from *Aspergillus niger*. The pH of STARGEN 001 enzyme ranged from 4.0 - 4.5. The specific gravity of STARGEN 001 enzyme is 1.10-1.15 g/mL. The recommended temperature for STARGEN 001 enzyme is 20-40°C. The minimum activity of STARGEN 001 enzyme is 456 GSHU/g. GSHU is defined as Granular Starch Hydrolyzing Units. The enzyme activity was determined by reaction at 37°C with soluble potato starch (1%) that was buffered with sodium acetate (pH 4.4). Aliquots were taken after 10 min for determining the amount of D-glucose released. The glucose was determined by using dinitrosalicylic acid method (Miller, 1959). The enzyme activity obtained was 3736 unit/g starch. The enzyme activity units (GSHU) are given as provided by the enzyme manufacturers. The assay protocol for determining enzyme activity can be obtained from the enzyme manufacturers.

Fractionation: Fractionation of wheat starch was performed according to the procedure described by Manelius *et al*. (1997). The starch was suspended in ~3 parts of water (w/v) and the large granules (A) were allowed to precipitate by sedimentation for 60 min. The supernatant was removed and the precipitate re-suspended in water. The sedimentation was then repeated several times until the precipitate was practically free from small granules (B) when observed in a light microscope. The A granules were washed in methanol and dried with acetone. The supernatant from each sedimentation was centrifuged at low speed (8g, 8 min) to remove large and intermediate size granules. The remaining small granules in the supernatant were then collected by centrifugation at high speed (2457g) washed in methanol and dried with acetone.

Starch hydrolysis: The starch (dry basis) slurry (25% w/v) was prepared in 400 mL of sodium acetate buffer. The enzyme (3736 unit/g starch) was added (1% w/v) into the samples. Samples were then incubated in an incubator shaker (JEIO Tech, SI-600R, Seoul, Korea) at 35°C at a speed of 150 rpm. After 24 h, hydrolysis was stopped by adjusting the pH to 1.5-1.6 with 2 M HCl. This step was done quickly to minimize further hydrolysis of the starch. Preliminary experiments have established that the enzyme deactivation method does not appear to cause significant starch hydrolysis. The pH of starch suspensions was adjusted back to a pH of 5-6 by washing and filtering the starch with distilled water. Starch residues were collected and dried at 40°C for 2 days.

Determination of Dextrose Equivalent (DE): The reducing sugar value was measured using the dinitrosalicylic acid method (Miller, 1959) to determine its Dextrose Equivalent (DE). For sampling intervals, a small portion of aliquot was withdrawn from each batch of starch slurry at various time intervals up to 24 h hydrolysis time. The absorbance was measured at 504 nm by using a UV/Visible spectrophotometer (UV-160A, SHIMADZU, Kyoto, Japan). Glucose ($C_6H_{12}O_6$) was used as a standard and the calibration curve was shown in Appendix A. Each analysis was performed in duplicate. DE was calculated as follows:

DE = (g reducing sugar expressed as glucose)/(g dry solid weight) ×100%

Scanning Electron Microscopy (SEM): Microstructure of starch granule was viewed with a field emission scanning electron microscope (FESEM Leo Supra 50VP, Carl-Ziess SMT, Oberkochem, Germany). The starch granules were stuck on aluminum specimen stubs with double-sided adhesive tape and sputter with a 20-30 nm layer gold using Sputter Coater [Polaron (Fisons) SC515, VG Microtech, Sussex, UK]. The accelerating voltage of the SEM is 5kV.

Determination of amylose content: Amylose content of each sample and raw starch was determined in

triplicate according to the procedure described by McGrance *et al*. (1998) with minor modification. Pure potato amylose and amylopectin (Sigma Chemical Company, Steinheim) were used as the standards. The results were expressed on a dry basis. Starch (0.1 g, dry basis) was accurately weighed and dissolved by heating in dimethyl sulphoxide (DMSO) for 15 min on a hot plate at 85°C while stirring continuously with a magnetic stirrer bar. After the solution had dissolved, it was diluted to 25 ml in a volumetric flask with deionized water. An aliquot (1 mL) of this solution was diluted with 50 ml of deionized water. Five ml iodine (0.0025 mol/L) in potassium iodide (0.0065 mol/L) was added with mixing and the absorbance was read at 600 nm using a UV/Visible spectrophotometer (UV-160A, SHIMADZU, Kyoto, Japan). Samples were left for 15 minutes after the addition of iodine before taking the readings on the spectrophotometer.

Swelling and solubility: Swelling power and solubility of starch were determined in triplicate, using the method described by Schoch (1964). Starch (100 mg, dry basis) was accurately weighed in a 50 mL ependorf tube and 10 mL of distilled water was added. The tube was placed in a water bath at 80°C for 30 min until the suspension was translucent. The solution was centrifuged (2328×g, 15 min) and then, the supernatant was carefully discarded. The swollen starch sediment was weighed. To determine the amount of soluble starch, an aliquot (5 mL) of the supernatant was dried overnight in an oven at 110°C. Swelling power was the ratio in weight of the wet sediment to the initial weight of dry starch. The solubility was the ratio in weight of the dried supernatant to the initial weight of starch.

X-ray diffraction: Crystallinity patterns of starch granules were examined by X-ray diffraction, as described by Lauro *et al*. (1999). The dried starches were conditioned overnight at room temperature in 100% Relative Humidity (RH). The starches were scanned by X-ray diffractometer (Diffractometer D5000, SIEMENS, Karlsruhe, Germany). Diffractograms were recorded in the reflection mode in the angular range of 4-40° (2θ) with a rate of 0.05 deg/s. The Cu K_{α}-radiation (λ 1.5406 Å), which was generated at 40 kV and 30 mA, was made monochromatic using 15 μm of Ni-foil. Scattered radiation was detected using a proportional detector.

Particle size distribution: Particle size distributions of the granules were determined with a low angle laser light scattering (Mastersizer S, Malvern Instruments Malvern, UK).

Statistical analysis: All tests were performed at least in duplicate. Analysis of variance (ANOVA) was performed using the Duncan's multiple range tests to compare treatment means (Steel and Torrie, 1960). Significance was defined at p<0.05.

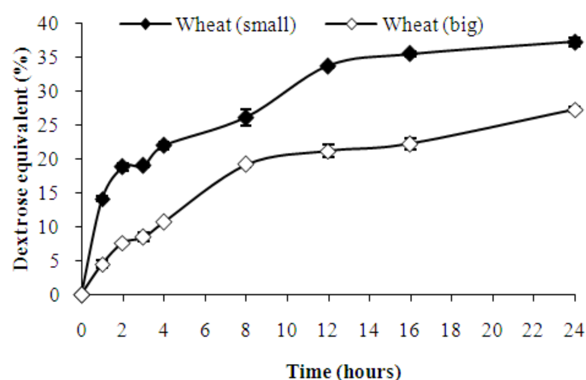


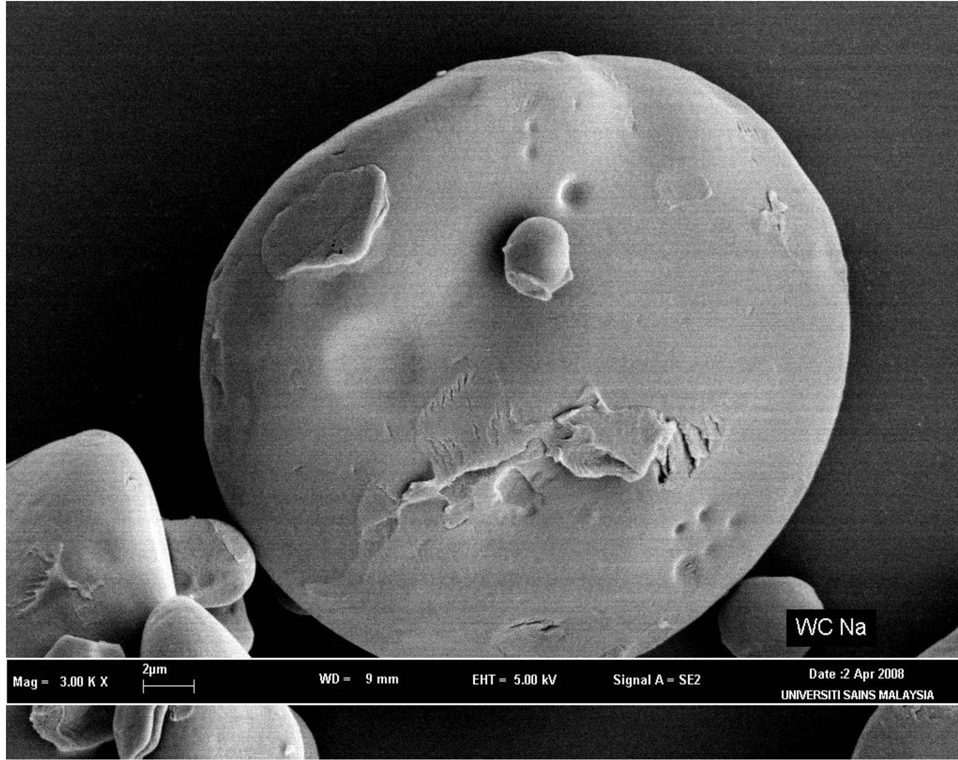
Fig. 1: Hydrolysis profile of wheat starch (with different size) below gelatinization temperature (35°C) for 24 h. Data points are mean±standard deviation (n = 3)

RESULTS AND DISCUSSION

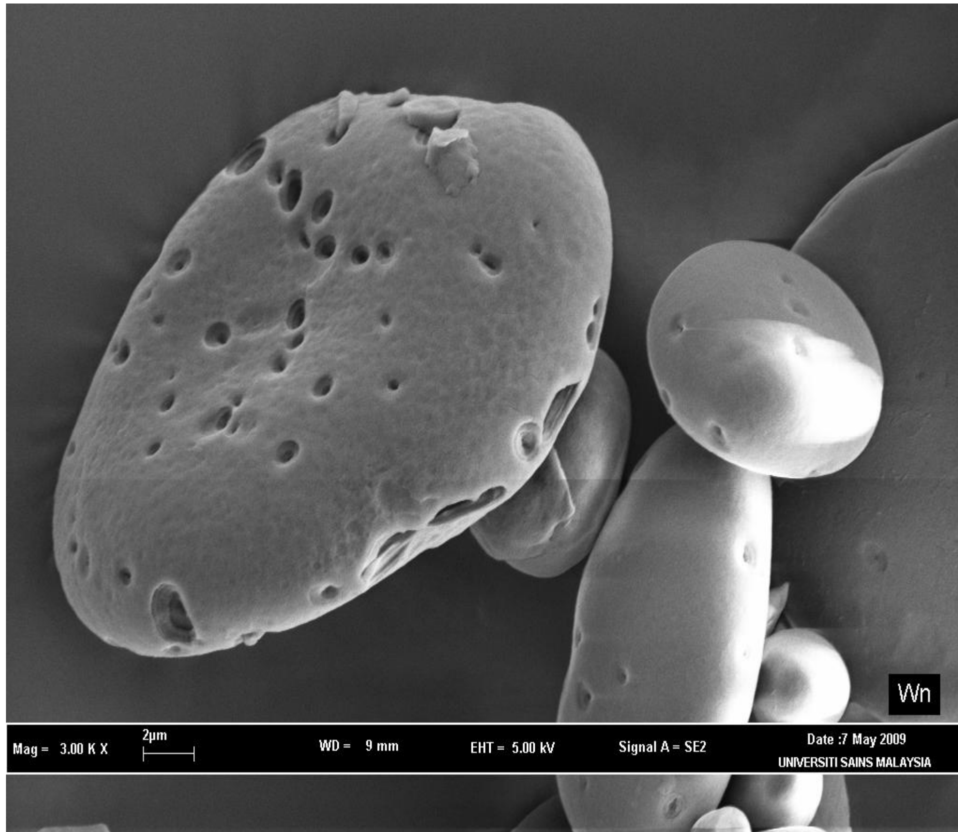
In the following discussion, the term 'A-type' refers to large wheat while 'B-type' refers to small wheat starch granules.

Hydrolysis profile: Hydrolysis profile of small and large wheat granules are shown in Fig. 1. According to the results, small wheat granules were more susceptible to enzymatic attack compared to their large counterparts with DE at 37 and 27%, respectively. This might due to the higher surface area per unit weight of small granule. Our result is in accordance with work by Manelius *et al*. (1997) who reported that small wheat granules were more efficient to be degraded by amylase than large granules. Kulp (1973) also reported that small wheat granules hydrolyzed faster with acid or enzyme than large granules. The hydrolysis process includes the diffusion of enzymes to the granule surface followed by adsorption and subsequent catalytic events (Colonna *et al*., 1992). Smaller granules, by virtue of their higher available surface area per unit mass, facilitate diffusion and adsorption of enzymes, accelerating the catalytic action.

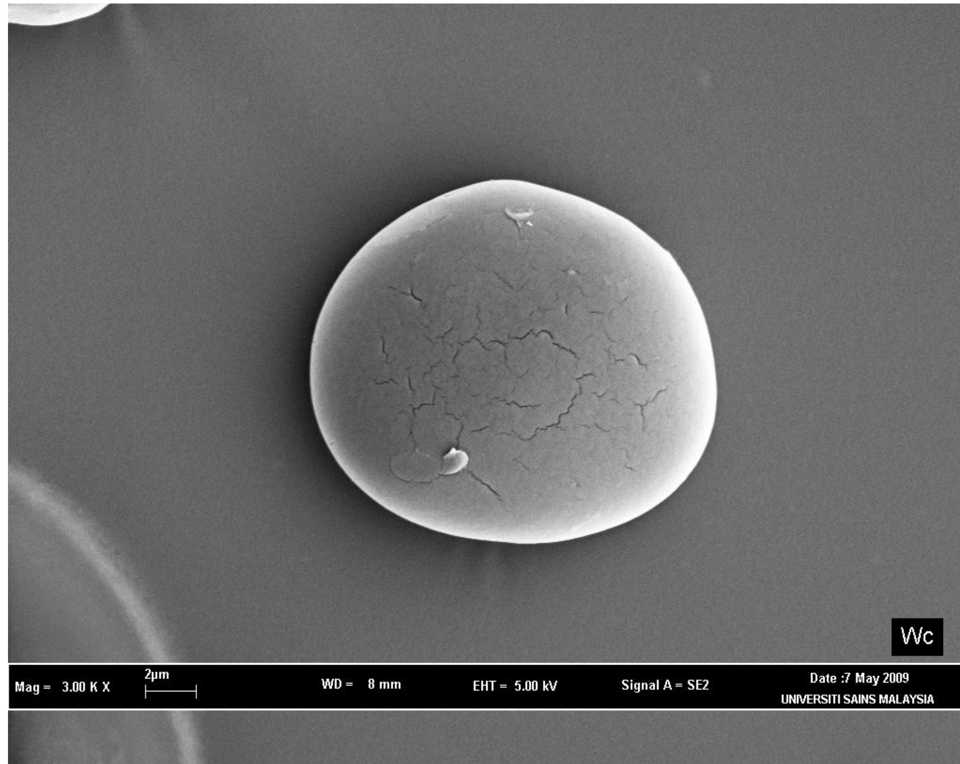
Enzymatic hydrolysis of a native starch is a solid-solution two phase reaction in which the enzyme needs first to diffuse to and adsorb on the solid substrate before catalyzing the cleavage of glycosidic linkages (Zhang *et al*., 2006). Diffusion of enzymes onto the starch surface and then inside the granules, therefore, may be rate limiting steps in enzymatic hydrolysis. Smaller granules, by virtue of their larger specific surface area (Fig. 1), facilitate adsorption of enzymes (Colonna *et al*., 1992) and thus are hydrolyzed more rapidly compared to larger granules (Fig. 2).



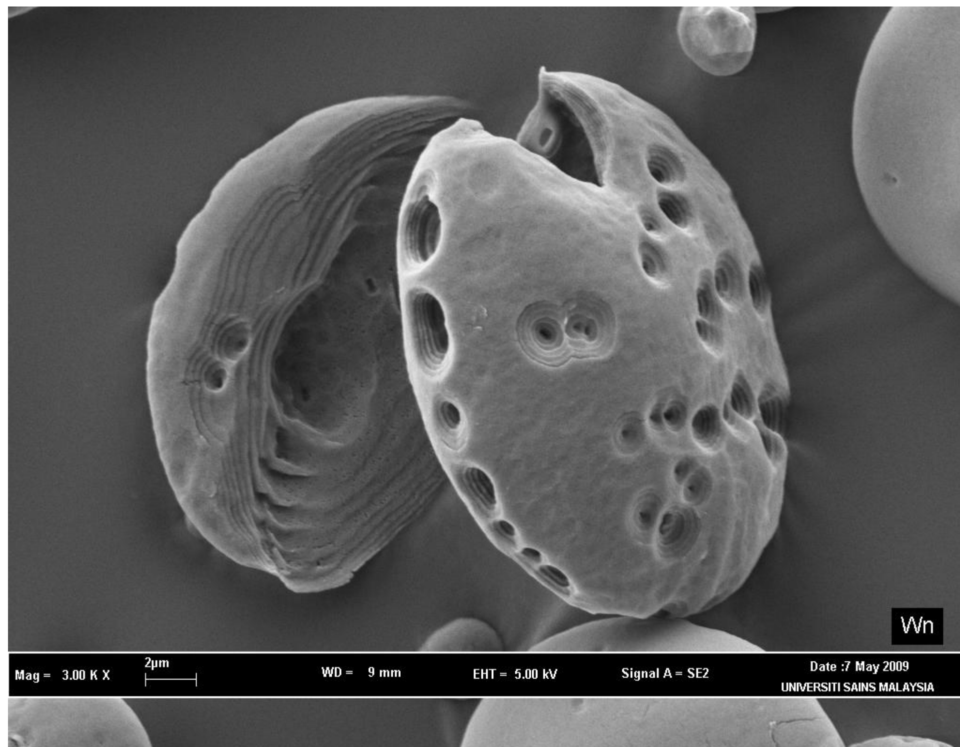
(a)



(b)



(c)



(d)

Fig. 2: SEM micrographs (3000×) for (a) Control large wheat (b) Hydrolyzed large wheat (c) Control small wheat (d) Hydrolyzed small wheat starches after 24 h of hydrolysis below gelatinization temperature (35°C) for 24 h (Scale bar = 2 μm)

Scanning Electron Microscopy (SEM): Micrographs of large (A) and small (B) wheat granules observed with SEM are shown in Fig. 2. The large granules are typically flat and posses a characteristic of equatorial groove, whereas small granules are rounded and our result is in accordance with study reported by Jane *et al*. (1994). The B-type granules appeared to be more irregular than the A-type and they were also more agglomerated. Control wheat starch especially the A type granule consist of pores which could be found along the equatorial groove. Fannon *et al*. (1992) reported that pores were found along the equatorial groove of large granules of wheat and barley starches, but not in small granules. These pores and pinholes are believed to facilitate the attack of enzymes during hydrolysis. Lindeboom *et al*. (2004) also claimed that no pinholes were detected in small granules. The erosion pattern of the B granules did not differ from the attack pattern on A granules. Both population possessed granules with roughened surface and clear holes of erosion after enzymatic attack. The typical “growth ring structure” was more clearly seen in the A-granules than in B granules (Fig. 2). Manelius *et al*. (1997) also reported the same pattern of enzymatic attack and showed that the A granules seem to have thicker outer layer that was denser than the inner part where the “growth ring” were more separated from each other. Some granules were extensively degraded, others were completely intact.

According to MacGregor and Morgan (1984), during hydrolysis of wheat, the large granules obtain erosion holes especially along the equatorial groove, whereas the small granules are protected from attack by surrounding protein layers in the endosperm. If the wheat starch is fractionated, the small granules, however, hydrolyzed faster than the large granules. This report is in accordance with the hydrolysis profile, where small granules showed higher susceptibility to enzymatic attack compared to large granules. Small granules of wheat (Fig. 2d) showed a lot of porous structure and surface erosion indicates that the enzyme had extensively attack the small granules. When wheat starch was treated with enzymes, several patterns of attack on the granules were observed (Gallant *et al*., 1973). Besides an endocorrosion through holes at the groove and other parts of the surface, exocorrosion and radial degradation between the characteristic interiors “growth ring” appeared.

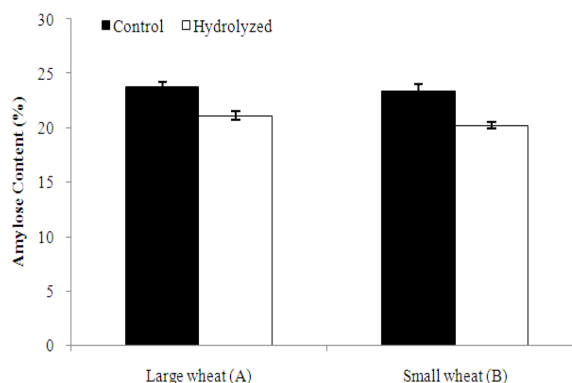


Fig. 3: The amylose content of large (A) and small (B) wheat starch after 24 h of hydrolysis at 35ºC. Bars are mean±standard deviation (n = 3). Different letters on top of each bar indicates significant difference ($p<0.05$) between bars

Amylose content: Figure 3 shows the amylose content of small and large granules of wheat starch for control and hydrolyzed starch. The larger granules had higher amylose content but insignificantly compared to small granules, as previously reported by Peng *et al*. (1999). Thus, there are differences not only in size but also in structure between the A- and B- type granules of wheat, which might result in different properties of hydrolyzed starches. Morrison (1989) reported that amylose content was higher in large granules while others reported the same amylose content in both small and large granules (Evers, 1974).

After hydrolysis, both granules showed significant decreased in amylose content. However, small granules (B) showed higher decrement and this is in accordance with hydrolysis profile where small granules are more extensively degraded compared to larger granules.

Swelling and solubility: Swelling power of A- and B-granules of control and hydrolyzed starches are shown in Table 1. The results show that swelling power of A granules was higher than the B granules. Hence, the A granules absorb more water than the B granules. The differences in the swelling power are partly affected by hydrocarbon chain of internal lipids, which suppress hydration of amorphous region in starch granules (Tester and Morrison, 1990). Therefore, the B type granules that contain higher amount of amylose lipid complex swell less than that of the A type granules.

Table 1: The swelling power and solubility of starches after 24 h of hydrolysis at 35°C

Sample		Swelling power (g/g)	Solubility (%)
Wheat	Control A	9.2 ± 0.1^b	2.5 ± 0.2^a
	Hydrolyzed A	9.0 ± 0.2^b	2.5 ± 0.1^a
	Control B	8.5 ± 0.4^a	2.5 ± 0.3^a
	Hydrolyzed B	8.4 ± 0.1^a	2.3 ± 0.1^a

Comparison within the column was shown in the table with the data written as mean±standard deviation (n = 3). Means within the same column not followed by the same letter are significantly different at $p<0.05$ level of significance, according to Duncan’s Multiple-Range Test

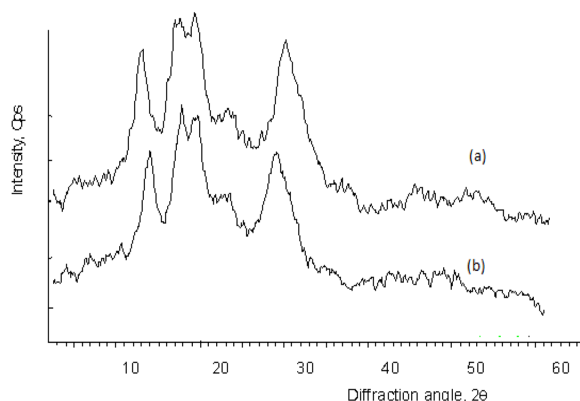


Fig. 4: X-ray diffraction pattern of (a) Control and (b) Hydrolyzed wheat starch after 24 h of hydrolysis

Table 2: Particle-size distribution and mean diameter of the control and hydrolyzed wheat starches after 24 h of hydrolysis

Sample		Mean diameter (μm)
Wheat	Control	45.18±0.10[c]
	Hydrolyzed	15.56±1.20[a]
Wheat (large)	Control	44.10±0.20[c]
	Hydrolyzed	15.51±0.50[a]
Wheat (small)	Control	20.13±0.40[b]
	Hydrolyzed	14.90±0.60[a]

Comparison within the column was shown in the table with the data written as mean±standard deviation (n = 3). Means within the same column not followed by the same letter are significantly different at $p<0.05$ level of significance, according to Duncan's Multiple-Range Test

Hydrolyzed starches showed no significant changes compared to control starch. This shows that the hydrolyzed granules still preserve its integrity and able to swell. This could be due to the un-uniformity of hydrolysis where only some of the granules are being hydrolyzed, while the rest are still remaining intact (Fig. 2).

X-ray diffraction: The X-ray diffraction pattern of the A- and B-type wheat starch granules showed no significant changes; therefore only results for control and hydrolyzed wheat starch (large granules) are shown in Fig. 4. Evidently, the differences in granule size did not alter the X-ray diffraction pattern. Wheat starch shows the A-pattern, with strong reflections at 2θ about 15° and 23° and an unresolved doublet at 17° and 18° 2θ. Hydrolyzed wheat showed the same X-ray pattern as control wheat, suggesting that hydrolysis occurred in amorphous granule.

Particle size distribution: Particle size distribution and mean diameter of small and large wheat starch for control and hydrolyzed wheat granules are shown in Table 2. From the results, mean diameter of control large granules is around 44 μm and control small granule is around 20 μm. It is widely acknowledged that, wheat contain two type of starch granules, i.e., large; A type granules >10 μm and small; B type granule ≤10 μm (Eliasson and Karlsson, 1983). The results showed that the mean diameter of small granules is a bit higher (20 μm) and this could due to the agglomeration of the small granules. Furthermore, our result was supported by Raeker *et al*. (1998), who reported that B-type granules were usually highly agglomerated. In addition, during SEM observation, we found that some large and small granules were still mixed, which means that the granules from the wheat were not perfectly separated. According to Raeker *et al*. (1998), the accuracy of granule size distribution is dependent on both starch isolation methods and size determination technique.

After hydrolysis, both hydrolyzed large and small granules showed significant decreased in mean diameter compared to their respective control. This is understandable as the enzyme hydrolyzed the starch, diameter of the granules would decrease. However, hydrolyzed B-type granules showed higher decrement compared to hydrolyze A-type granules, indicating that hydrolysis occurred more extensively in small granules. This result is in accordance with hydrolysis profile where small granules showed higher susceptibility to enzymatic attack compared to large granules.

CONCLUSION

The physicochemical properties of native and hydrolyzed starches from the A-type wheat starch granules were significantly different from those of the B-type granules. The small starch granules (B) were more readily attacked by the enzymes than the large granules (A) with DE attained at 37 and 27%, respectively. This could be the result of the small differences in the composition of the two granules population. In addition, the higher surface area per unit weight of small granule starch also contributes to the higher susceptibility of small granules. Therefore, it is proven that small granules are easier to be hydrolyzed compared to larger granules by granular starch hydrolyzing enzyme.

REFERENCES

Colonna, P., V. Leloup and A. Buléon, 1992. Limiting factors of starch hydrolysis. Eur. J. Clin. Nutr., 46(Suppl. 2): S17-S32.

Eliasson, A.C. and R. Karlsson, 1983. Gelatinization properties of different size classes of wheat starch granules measured with differential scanning calorimetry. Starch-Starke, 35(4): 130-133.

Evers, A.D., 1974. The development of the grain of wheat. Proceeding of the 4th International Congress of Food Science and Technology. Madrid, 1: 422-431.

Fannon, J.E., R.J. Hauber and J.N. BeMiller, 1992. Surface pores of starch granules. Cereal Chem., 69: 284-288.

Gallant, D.J., A. Derrien, A. Aumaitre and A. Guilbot, 1973. *In vitro* degradation of starch. Studies by transmission and scanning electron microscopy. Starch-Starke, 25: 56-64.

Jane, J.L., T. Kasemsuwan, S. Leas, H. Zobel and J.F. Robyt, 1994. Anthology of starch granule morphology by scanning electron microscopy. Starch-Starke, 46(4): 121-129.

Kulp, K., 1973. Characteristics of small-granule starch of flour and wheat. Cereal Chem., 50: 666-679.

Lauro, M., P.M. Forssell, M.T. Suortti, S.H.D. Hulleman and K.S. Poutanen, 1999. α-amylolysis of large barley starch granules. Cereal Chem., 76(6): 925-930.

Li, J.H., T. Vasanthan, R. Hoover and B.G. Rossnagel, 2004. Starch from hull-less barley: V. In-vitro susceptibility of waxy, normal, and high-amylose starches towards hydrolysis by alpha-amylases and amyloglucosidase. Food Chem., 84(4): 621-632.

Li, W.Y., S.H. Yan, Y.P. Yin, Y. Li, T.B. Liang, F. Gu, Z.M. Dai and Z.L. Wang, 2008. Comparison of starch granule size distribution between hard and soft wheat cultivars in Eastern China. Agr. Sci. China, 7(8): 907-914.

Lindeboom, N., P.R. Chang and R.T. Tyler, 2004. Analytical, biochemical and physicochemical aspects of starch granule size, with emphasis on small granule starches: A review. Starch-Starke, 56(3-4): 89-99.

MacGregor, A.W. and J.E. Morgan, 1984. Structure of amylopectin from large and small starch granules of normal and waxy barley. Cereal Chem., 61: 223-233.

Manelius, R., Z. Qin, A.K. Åvall, H. Andtfolk and E. Bertoft, 1997. The mode of action on granular wheat starch by bacterial α-amylase. Starch-Starke, 49(4): 142-147.

McGrance, S.J., H.J. Cornell and C.J. Rix, 1998. A simple and rapid colorimetric method for the determination of amylose in starch products. Starch-Starke, 50(4): 158-163.

Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem., 31(3): 426-428.

Morrison, W.R., 1989. Uniqueness of Wheat Starch. In: Pomeranz, Y. (Ed.), Wheat is Unique. American Association of Cereal Chemists, St. Paul, Minnesota, pp: 193-214.

Peng, M., M. Gao, E.S.M. Abdel-Aal, P. Hucl and R.N. Chibbar, 1999. Separation and characterization of A- and B-type starch granules in wheat endosperm. Cereal Chem., 76(3): 375-379.

Raeker, M.O., C.S. Gaines, P.L. Finney and T. Donelson, 1998. Granule size distribution and chemical composition of starches from 12 soft wheat cultivars. Cereal Chem., 75(5): 721-728.

Robertson, G.H., D.W. Wong, C.C. Lee, K. Wagschal, M.R. Smith and W.J. Orts, 2006. Native or raw starch digestion: A key step in energy efficient biorefining of grain. J. Agr. Food Chem., 54(2): 353-365.

Schoch, T.J., 1964. Swelling Power and Solubility of Granular Starches. In: Whistler, R.L. (Ed.), Methods in Carbohydrate Chemistry. Vol. 4, Academic Press, London.

Singh, H. and S.K. Soni, 2001. Production of starch-gel digesting amyloglucosidase by *Aspergillus oryzae* HS-3 in solid state fermentation. Process Biochem., 37(5): 564-573.

Slaughter, S.L., P.R. Ellis and P.J. Butterworth, 2001. An investigation of the action of porcine pancreatic alpha-amylase on native and gelatinised starches. Biochem. Biophys. Acta, 1525(1-2): 29-36.

Tester, R.F. and W.R. Morrison, 1990. Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose, and lipids. Cereal Chem., 67(6): 551-557.

Topping, D.L., J.M. Gooden, I.L. Brown, D.A. Biebrick, L. McGrath, R.P. Trimble, M. Choct and R.J. Illman, 1997. A high amylose (*Amylomaize*) starch raises proximal large bowel starch and increases colon length in pigs. J. Nutr., 127(4): 615-622.

Weurding, R.E., A. Veldman, W.A. Veen, P.J. van der Aar and M.W. Versteqen, 2001. *In vitro* starch digestion correlates well with rate and extent of starch digestion in broiler chickens. J. Nutr., 131(9): 2336-2342.

Zhang, G., Z. Ao and B.R. Hamaker, 2006. Slow digestion property of native cereal starches. Biomacromolecules, 7(11): 3252-3258.