Enzymatic hydrolysis of lotus rhizome starch using α-amylase and glucoamylase

LI GUO

Summary

To study the susceptibility of lotus root starch to digestive enzymes and its potential impact on glycemic response, enzyme kinetics and in vitro digestibility of the granular, gelatinized and retrograded starches were analysed. The results showed that the digestion rate coefficient values of the granular, gelatinized and retrograded starches were 4.6×10^{-3} min^{-1}, 9.8×10^{-3} min^{-1} and 2.3×10^{-3} min^{-1}, respectively. Compared to the granular starch, content of rapid digestible starch (RDS) increased by 39.0 %, content of slowly digestible starch (SDS) and resistant starch (RS) decreased by 9.6 % and 15.0 % after gelatinization, respectively. While content of RDS decreased by 21.1 %, content of SDS and RS increased by 2.1 % and 20.8 % after retrogradation, respectively. As for glycemic index (*GI*) and hydrolysis index (*HI*), *GI* (70.57) and *HI* (56.21) of the gelatinized starch were higher than *GI* (66.63) and *HI* (49.03) of the granular starch, and *GI* (57.83) and *HI* (33.01) of the retrograded starch. The results provide an interesting information about exploring novel and slow digestible foods made of lotus root starch for potential health benefits.

Keywords

lotus root starch; digestibility; α-amylase; glucoamylase

Lotus (*Nelumbo nucifera*) is a well-known medicinal plant widely cultivated in Asian countries, including China, Korea and Japan. Among the leaf, root, seed and flower of lotus, the root is used as a popular vegetable and can be eaten roasted, pickled, as dried slices or fried. It has many functional properties including antidiarrheal properties, antimicrobial activities, antifungal and anti-yeast activities, hypotension effect, hypocholesterolemic effect, hypoglycemic activity, psychopharmacological activity, alleviation of hepatic steatosis and diuretic activity [1]. These properties are directly related with the components in the root. Zhong et al. [2] and Zhu [3] reported that the fresh roots contains water (77.9–89.0 mg·kg^{-1}), starch (10–20 mg·kg^{-1}), proteins (1–2 mg·kg^{-1}), dietary fibres (1.2 mg·kg^{-1}), minerals and vitamins (1.25–1.55 mg·kg^{-1}), flavonoids (0.51 mg·kg^{-1}) as well as phenolic compounds, antioxidants, amino acids and fat. Based on this, starch is an important component in roots. Nowadays, lotus root starch is isolated from lotus root and widely consumed as a high-quality and valuable "healthy" food in China [2, 4].

Starch is hydrolysed to glucose by α-amylase and mucosal α-glucosidase in the human gastrointestinal tract [5, 6]. Based on enzymatic resistance, of starches are divided to rapid digestible starch (RDS), slow digestible starch (SDS) and resistant starch (RS) [7]. RDS refers to the starch fraction rapidly digested to glucose, within 20 min incubation, SDS is the starch being hydrolysed within 20–120 min incubation, and RS is the starch fraction not being hydrolysed after 120 min incubation, or can be never digested and leaves the body undigested. RDS contributes to the postprandial rise in blood glucose levels and insulin response, which is associated with some diseases including type II diabetes, obesity and cardiovascular disease [8]. So, it is interesting and important to study the relationship between lotus root starch and functional properties of lotus root.

For many years, the physicochemical properties of lotus root starch were widely reported, but digestibility of lotus root starch remains unclear. For example, starch granules are elongated in shape with a larger size, and some starch granules are oval and polygonal in shape with a smaller size [9]. The average particle sizes (the long axis length) are 26.63 μm, 17.86 μm and 34.42 μm for

Li Guo, School of Food Sciences and Engineering, Qilu University of Technology, No. 3501 Daxue Road, Changqing District, 250353 Jinan, China. Tel.: +86-531-89631162; fax: +86-531-89631195, e-mail: guolizhuyuer@163.com

total starch, oval starch and elongated starch, respectively. The starch granule contains 23.9% amylose and exhibits a typical C-type X-ray diffraction (XRD) pattern. The amylose content (23.9 mg·kg^{-1}) is higher than that (17.4 mg·kg^{-1}) reported by SUZUKI et al. [8] and lower than that (30.6 mg·kg^{-1}) reported by ZHONG et al. [2]. In addition, ZHONG et al. [2] indicated that lotus starches show a B-type XRD pattern, while LIN et al. [10] reported that the lotus starch exhibits a C-type XRD pattern.

Thus, it is important to investigate the susceptibility of lotus root starch to digestive enzymes and its potential impact on glycemic response in human system. The first objective of this study was to investigate the susceptibility of lotus root starch to α-amylase by examining the enzyme kinetics using the first-order model and Lineweaver-Burk plots. The second objective was to examine the digestibility of the granular, gelatinized and retrograded starches in an in vitro system in order to determine the mechanisms of lotus root starch digestion.

MATERIALS AND METHODS

Lotus root powder was from Zhoushi Food (Guangxi, China). The details about it are as follows: average particle size expressed as $D_{4,3}$ (i.e., mean volume diameter; 16.791 ± 0.014373 μm), colour (gray and white), moisture content 0.986 ± 0.001 g·kg^{-1} and total carbohydrate content 9.010 ± 0.001 g·kg^{-1}, sodium content 0.004 ± 0.001 g·kg^{-1}. Lotus root starch was isolated and purified according to the procedure of ZHONG, et al. [2]. α-Amylase from porcine salivary glands (EC 3.2.1.1, 25 U·mg^{-1}), α-amylase from porcine pancreas (EC 3.2.1.1, 37 U·mg^{-1}), glucoamylase from *Aspergillus niger* (EC 3.2.1.3, 100 U·mg^{-1}), porcine pepsin (EC 3.4.23.1, 3000 U·mg^{-1}), porcine pancreatin (EC 232-468-9, 8 × USP·g^{-1}), where USP stands for United States Pharmacopeia and specifies amylase, protease and lipase only, and isoamylase (EC 3.2.1.68, 3000000 U·mg^{-1}) from *Pseudomonas amylodermosa* were from Sigma-Aldrich (St. Louis, Missouri, USA). Activity was defined by the manufacturer. The glucose oxidase-peroxidase (GOPOD) assay kit was from Megazyme International (Wicklow, Ireland). D-(+)-Glucose monohydrate standard and the reagents used for quantification of reducing sugars were from Sigma-Aldrich.

Granule lotus root starch characteristics

Starch composition

The starch content was determined according to a previously published method [5]. Amylose and amylopectin of lotus root starch were fractionated according to the protocols described by NAGULESWARAN et al. [11]. The amylose content was determined using an iodine colorimetric method [12]. Lotus root starch was analysed for the contents of lipids (method 945.16), proteins (method 992.15; N × 5.95), ash (method 920.153) and moisture (method 985.14) according to the protocols of the Association of Official Analytical Chemists [13]. Based on glucose content, the free sugar was measured using the 3,5-dinitrosalicylic acid (DNS) colorimetric method [6]. In this study, data on the composition expressed per kilogram of the lotus root starch are listed below: starch 9.342 ± 0.001 g·kg^{-1} (amylose 2.029 ± 0.001 g·kg^{-1}; free sugar 0.498 ± 0.002 g·kg^{-1}); water 0.637 ± 0.002 g·kg^{-1}; protein 0.012 ± 0.003 g·kg^{-1}; lipid 0.002 ± 0.001 g·kg^{-1}; ash 0.007 ± 0.001 g·kg^{-1}.

Starch granule size distribution

Particle size analysis was determined by using a laser light scattering particle size analyzer (Mastersizer Hydro 2000MU, Malvern Instruments, Malvern, United Kingdom). Granular lotus root starch was suspended in distilled water and stirred at 50 Hz. A general analysis model was used with particle refractive and absorption indices of 1.53 and 0.01, respectively, and the refractive index of water as the dispersant was 1.33. Starch particles were examined within the range from 0.02 μm to 2000 μm. The obscuration in all the measurements ranged from 9 % to 13 %. Particle size was defined in terms of the volume weighted mean $D_{4,3}$, 10th percentile $d_{0.1}$, 50th percentile or median $d_{0.5}$, 90th percentile $d_{0.9}$ and surface weighted mean $D_{3,2}$ used to determine the specific surface area (*SSA*) expressed in square metres per gram assuming spherical granules of uniform density. Analyses were done in triplicate and results were expressed as mean ± standard deviation.

Starch granule morphology

Granule morphology of the granular and partially hydrolysed lotus root starches were studied by using a Hitachi scanning electron microscope (S-4800; Hitachi High Technologies, Tokyo, Japan) at an accelerating potential of 10 kV. The partially hydrolysed granules were collected from the in vitro digestion at 20 min and 120 min. Dry starch samples were brushed onto the surface of double-sided carbon adhesive tape mounted on an aluminum stub and then coated with a thin film (20 nm) of gold in an argon atmosphere.

Starch granule crystallinity

The moisture content of the powdered samples was adjusted to 2.00 ± 0.005 g·kg^{-1} by placing them in a desiccator over saturated K_2SO_4 (25 °C, a common and constant temperature to obtain a constant water activity a_w = 0.98) for 10 days until the moisture content was constant. The crystallinity of granular lotus root starch and partially hydrolysed granules obtained from in vitro digestion at 20 min and 120 min were analysed using an X-ray diffractometer. The diffractograms of samples were produced by using a Theta/Theta rotating anode X-ray diffractometer (Rigaku, Tokyo, Japan) operating at 40 kV and 200 mA at 20 °C. The scanning region of the diffraction angle (2θ) was from 4° to 40° at a step of 0.02° and scanning speed of 4° per minute. CuK_a-radiation (λ = 0.15406 Å) was selected using a graphite monochromator. A divergence slit of 1° and a receiving slit of 0.3 mm were chosen. The crystallinity was defined as the percentage of crystalline material present in the retrograded starch (absolute percentage). The percentage of crystalline material was calculated as the ratio of the area of the crystalline reflections to the overall area, using the method of Hermans and Weidinger [14]. The total area and crystalline area were measured using Jade software (Jade Software, Cardiff, United Kingdom). Analyses were done in triplicate and results are expressed as mean ± standard deviation.

Susceptibility of lotus root starch to digestive enzymes

Enzyme kinetics of α-amylase

Granular, gelatinized and retrograded starches (0–25 mg·ml^{-1}) and porcine pancreatic α-amylase (10 U·ml^{-1}) were mixed in a phosphate buffered saline (PBS, Waltham, Massachusetts, USA) with a pH of 6.8 and then incubated in a water bath controlled at 37 °C with magnetic stirring (ZLD-300, Zonce Machinery, Shanghai, China) under a constant rotating speed (50 Hz). After 10 min, the slurry was placed in boiling water for 20 min to inactivate α-amylase. The amount of reducing sugar was determined by using DNS colorimetric assay [5]. Michaelis-Menten equation of enzyme-catalysed reaction of single substrate is [15]:

$$\frac{1}{V} = \frac{1}{V_m} + \frac{K_m}{V_m}\frac{1}{[S]} \quad (1)$$

where K_m is the Michaelis constant, V_m is the maximum velocity of the reaction achieved when the enzyme active sites in the sample are all complexed with substrate all the time, and S is the substrate concentration. Thereby, V_m and K_m values can be obtained from the intercept and slope of the Michaelis-Menten plot, respectively.

Susceptibility of starches to α-amylase

Granular starch hydrolysis

Granular starch (500 mg, dry basis) was suspended in 50 ml of PBS with a pH of 6.8 containing 0.1 ml of 10% sodium azide with constant mixing (50 Hz). Porcine pancreatic α-amylase (10 U·ml^{-1}) was added to the starch slurry, and then was heated and held in a shaking water bath at 37 °C for 140 min. Aliquots (1 ml) of starch hydrolysates were taken at time intervals between 20 min and 140 min (20, 40, 60, 80, 100, 120 and 140 min) and immediately placed in boiling water for 20 min to inactivate the enzymatic activity. The aliquots were filtered through a membrane (pore size: 0.22 μm) to remove the non-reacted starch residues, and the supernatant was analysed for reducing sugars. The degree of hydrolysis was defined as the reducing sugars generated in supernatant, expressed as milligrams of maltose equivalents released per kilogram (dry weight) of starch. All analyses were carried out in triplicate.

Gelatinized starch hydrolysis

The granular lotus root starch (50 mg) was suspended in water (5 ml) in screw-cap tubes and heated in a boiling water bath with magnetic stirring for 30 min to gelatinize the lotus root starch, and then cooled to 37 °C. The above described assay of digestibility followed.

Retrograded starch hydrolysis

After gelatinization, gelatinized starch was cooled and stored at 4 ± 1 °C for 7 days. The above described assay of digestibility followed.

Simulation of in vitro digestion

A simulation of in vitro digestion is commonly used to predict the glycemic response in humans. When the digestion rate is constant, the digestion data fit in the first-order model Eq. 2 [16]:

$$C_t = 1 - e^{-kt} \quad (2)$$

where C_t is the starch digested (expressed as percentage) at incubation time t (in minutes), $(1-C_t)$ is the undigested starch remaining after incubation time t, and k is the digestion rate coefficient (in reciprocal minutes). The value of k is obtained from the slope of a linear-least-squares fit of the ln $(1-C_t)$ plot against t.

The in vitro starch digestion procedure outlined by Al-Rabadi et al. [17] was used with minor modifications. The method was carried out

in three successive steps. The first step was to mimic digestion in the mouth: each starch sample (1.0 g, dry basis) was treated with 20 ml of artificial saliva containing porcine salivary α-amylase in carbonate buffer with a pH of 7 at 37 °C for 10–15 s. This step was followed by enzymatic hydrolysis with 10 ml porcine pepsin solution at pH 2 using 0.02 mol·l^{-1} hydrochloric acid and incubation in a shaking water bath (SHA-C, Changzhou Guoyu Instrument, Changzhou, China) at 37 °C with constant mixing (50 Hz) for 30 min. The third digestion step simulating small intestinal conditions was performed with pancreatin, a mixture of primarily protease and pancreatic α-amylase, and glucoamylase. After adjusting the pH to 7.0 with 1 mol·l^{-1} sodium hydroxide, 70 ml pancreatin and glucoamylase solution (prepared by adding 2 mg pancreatin and 1 mg glucoamylase per millilitre of acetate buffer containing 0.2 g·l^{-1} sodium azide) was added to the mixed solution, and then the solution was incubated in a water shaking bath controlled at 37 °C for six time periods (0, 20, 40, 60, 120, 180 and 360 min). Zero time digestion was started at the beginning of the small intestinal simulation step because the small intestine is the main site of starch digestion. At the end of each incubation period, the hydrolysate was immediately placed in boiling water for 20 min to inactivate the enzyme activity and then the glucose concentration was measured using the GOPOD kit.

The values of RDS, SDS and RS were obtained using the following formulas according to the previous publication [7] and expressed in percent:

$$RDS = (G_{20} - FG) \times 0.9 \quad (3)$$

$$SDS = (G_{120} - G_{20}) \times 0.9 \quad (4)$$

$$RS = (TG - G_{120}) \times 0.9 \quad (5)$$

where G_{20} and G_{120} is content of glucose (in milligrams per kilogram) released during starch hydrolysis for 20 min and 120 min, respectively; *FG* is content of free glucose in starch (in milligrams per kilogram) and *TG* is content of total glucose released during the whole starch hydrolysis (in milligrams per kilogram). All analyses were carried out in triplicate.

Hydrolysis index and glycemic index

The standardized white wheat flour bread with glycemic index (*GI*) equal to 100 was used as a reference when evaluating the rate of starch hydrolysis in vitro and for the hydrolysis index (*HI*) calculation [18].

HI was calculated as the area under the hydrolysis curve (0–360 min) for starch samples and expressed as the percentage of the corresponding area for white bread [19].

GI was determined according to the equation described by Goñi et al. [20]:

$$GI = 39.71 + 0.549HI \quad (6)$$

Statistical analysis

The data were analysed by ANOVA, using the F-test to detect significant differences. Tukey's test was then performed to analyse pairwise comparisons of the means between groups where significant differences were detected. The significance level was set at 5%.

RESULTS AND DISCUSSION

Starch granule size distribution

Since granular lotus root starch contains little only small amounts of proteins and lipids, their effects on starch digestion will be negligible. The particle size parameters including the volume weighted mean diameter, surface area weighted mean diameter and specific surface area are presented in Tab. 1. The particle size distributions of lotus root starch were 9.587 μm, 16.289 μm and 28.266 μm for $d_{0.1}$, $d_{0.5}$ and $d_{0.9}$, respectively. Starch granules in this study were of a similar size as reported by Man et al. (26.63 μm) [9]. $D_{4,3}$ was 17.886 μm, corresponding to $D_{3,2}$, and *SSA* were 15.091 μm and 0.398 m^2·g^{-1}, respectively. Granule size plays an important role in affecting starch digestibility, in particular when particle size is below 270 μm [17]. Lotus root starch in this study had a particle size in the range of 5–50 μm, which suggests that particle size of lotus root starch may have a significant influence in its digestion behaviour.

Tab. 1. The particle size parameters.

Parameter	Value
$d_{0.1}$ [μm]	9.587 ± 0.015
$d_{0.5}$ [μm]	16.289 ± 0.002
$d_{0.9}$ [μm]	28.266 ± 0.001
Volume weighted mean $D_{4,3}$ [μm]	17.886 ± 0.016
Surface weighted mean $D_{3,2}$ [μm]	15.091 ± 0.010
Specific surface area *SSA* [m^2·g^{-1}]	0.398 ± 0.001

Data are expressed as mean ± standard deviation of triplicate measurements, means being significantly different at a 5% level.
Particle size measurements were reported as $d_{0.1}$, $d_{0.5}$ and $d_{0.9}$ on a volume basis that is the size of particle below which were 10%, 50% and 90% of the sample particles, respectively.

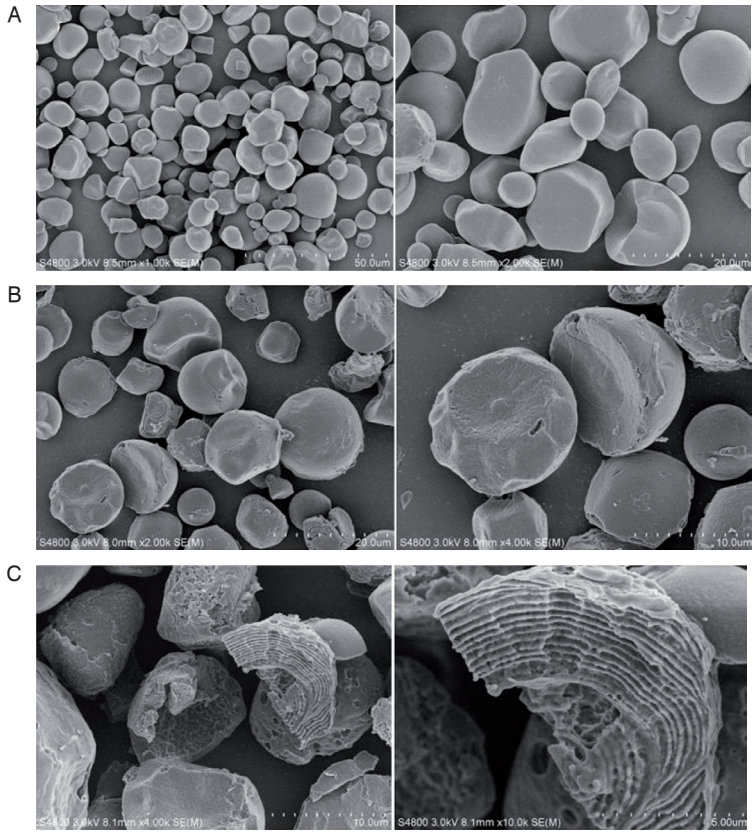

Fig. 1. Scanning electron microscope images of the hydrolysed granular starch.

A – the original starch granule; B – granular starch hydrolysed for 20 min; C –granular starch hydrolysed for 120 min.

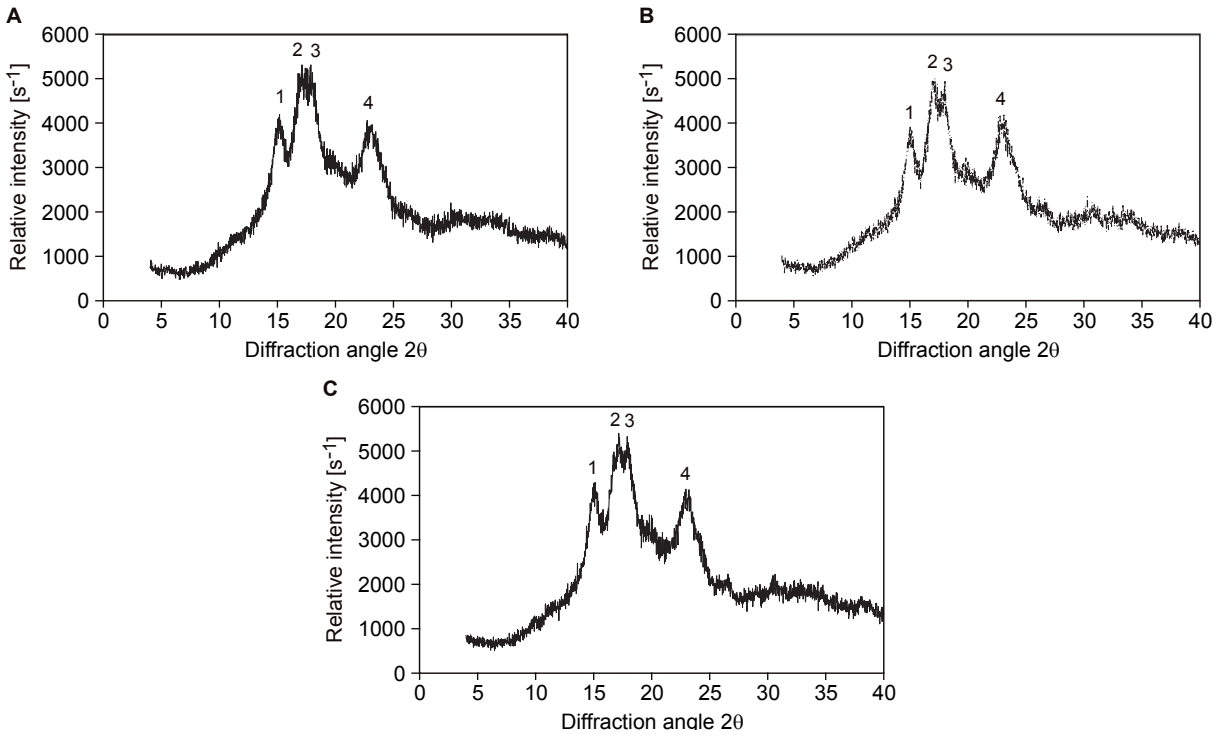

Fig. 2. X-ray diffraction patterns of the hydrolysed granular starch.

A – the original starch granule; B – granular starch hydrolysed for 20 min; C – granular starch hydrolysed for 120 min.

Characteristics of partially hydrolysed lotus root starch granules

Morphology

Fig. 1 presents the morphology of partially hydrolysed lotus root starch granules (hydrolysed for 0, 20 and 120 min). Compared to the granular lotus root starch (Fig. 1A), the starch granules did not show any apparent changes after 20 min of in vitro digestion, while small pores appeared on the surface and a few granule fragments appeared (Fig. 1B). The low degree of hydrolysis of the granular lotus root starch could be attributed to the morphological characteristics of the smooth granule surface (Fig. 1A). Hydrolysis of native starch granules was previously shown to begin at the granule periphery due to the presence of surface pores and channels pores, and channels were indicated to increase the effective surface area for fast enzyme diffusion [11, 12]. So, the smooth granule surface possessing few surface pores and channels may inhibit enzyme diffusion that, which may be responsible for the low degree of hydrolysis of lotus root starch granules. With further digestion (20–120 min), the pores on the surface became larger, and more broken granules with exposed interior and granule fragments were present. The erosion areas penetrated through several layers of the granule into its interior.

Crystallinity

Fig. 2 shows that the granular lotus root starch contained a C-type crystal with the major peaks 1 (15° 2θ), 2 (17° 2θ), 3 (18° 2θ) and 4 (23° 2θ) [21]. The relative crystallinity was 26.3 % for granular lotus root starch. Interestingly, hydrolysis did not change the crystalline type. However, there was an increase in crystallinity, that is, 29.1 % and 33.5 % for 20 min and 120 min, respectively. The change was primarily caused by extensive hydrolysis of the amorphous region of the starch granule, resulting in a relative increase of crystallinity [22].

Susceptibility of lotus root starch to digestive enzymes

Fig. 3 shows that K_m and V_m values of the granular starch were 9.985 mg·ml^{-1} and 2.282 mg·ml^{-1}·min^{-1}, respectively. The Michaelis-Menten equation of α-amylase reaction would be

$$V = \frac{2.282[S]}{9.985 + [S]} \quad (7)$$

For the gelatinized starch, K_m and V_m values were 6.606 mg·ml^{-1} and 1.959 mg·ml^{-1}·min^{-1}, respectively. The Michaelis-Menten equation of α-amylase reaction would be

$$V = \frac{1.959[S]}{6.606 + [S]} \quad (8)$$

For the retrograded starch, K_m and V_m values were 29.233 mg·ml^{-1} and 2.483 mg·ml^{-1}·min^{-1}, respectively. The Michaelis-Menten equation of α-amylase reaction would be

$$V = \frac{2.483[S]}{29.233 + [S]} \quad (9)$$

We observed that that gelatinization of starch had no significant influence on the susceptibility of lotus root starch to α-amylase. Lindeboom et al. [23] showed that the gelatinization property of starch is related to a variety of factors including the size, proportion and kind of crystalline organization, and ultrastructure of the starch granules. Based on the above data, crystals of the granular lotus root starch were of C-type, which is known to be more resistant to heat and hydrolysis than A-type starch [24]. Besides that, lotus root starch in this study contained high content of amylopectin (7.971 ± 0.01) g·kg^{-1}, which may obviously influence starch hydrolysis by amylases. Generally, amylopectin is more susceptible to enzymatic hydrolysis than amylose. The highly linear amylose chains yield sugars at a slower rate than the highly branched amylopectin [25]. However, it was reported that amylopectin molecules with a higher number of short chains with a greater branching degree have a more compact structure and high molecular density. Due to the high molar mass and small molecular size, amylopectin molecules are less susceptible to hydrolysis by amylases [26]. The mechanism of digestion of amylopectin branch

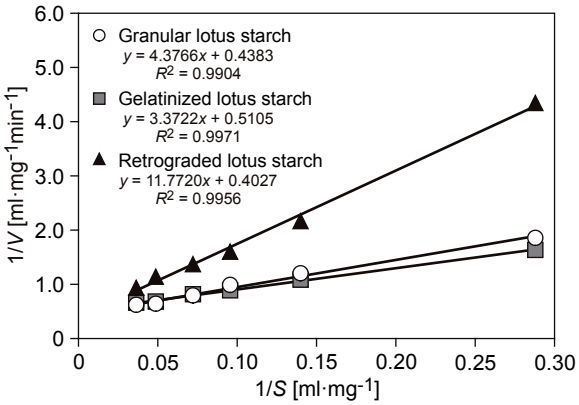

Fig. 3. Michaelis-Menten kinetics of the enzymatic hydrolysis.

A – The granular starch hydrolysed by α-amylase; B – gelatinized starch hydrolysed by α-amylase; C – retrograded starch hydrolysed by α-amylase.
V – reaction rate, *S* – substrate concentration.

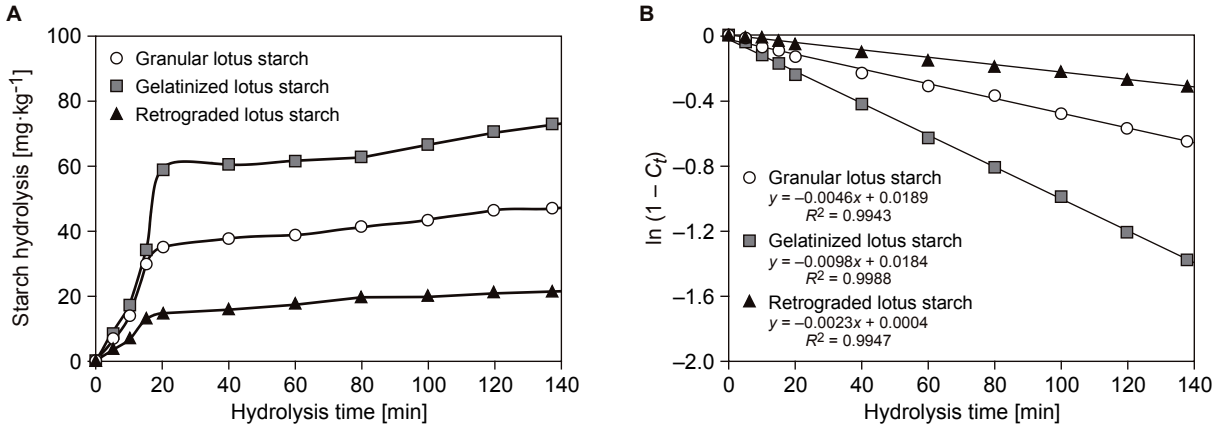

Fig. 4. Hydrolysis of lotus root starch using α-amylase.

A – time dependence of starch hydrolysis; B – fit of first-order kinetics.
C_t – the starch digested (expressed as percentage) at incubation time t (in minutes).

structures in the lotus root starch are not clear, the susceptibility of the amylopectin ultrastructure of the lotus root starch granule to amylolytic enzymes is studied in our present studies. Fig. 3 indicates that the K_m value of the retrograded starch was approximately 3-fold higher than the corresponding value of the granular starch, which implied that the retrograded starch was far less susceptible to α-amylase than the granular starch. This may be attributed to the tightly packed crystalline structure formed after cooling the gelatinized starch [3].

From Fig. 4A, it is evident that gelatinized starch was hydrolysed more rapidly than granular and retrograded starches at the initial stage. The hydrolysis of the granular, gelatinized and retrograded starches reached a plateau of 46.10 mg·kg^{-1}, 70.29 mg·kg^{-1} and 21.00 mg·kg^{-1} after 120 min, respectively. Fig. 4B presents the fit of first-order kinetics on the granular, gelatinized and retrograded lotus root starches. The digestion rate coefficient k values of the granular, gelatinized and retrograded starches were $(4.6 \pm 0.1) \times 10^{-3}$ min^{-1}, $(9.8 \pm 0.3) \times 10^{-3}$ min^{-1} and $(2.3 \pm 0.1) \times 10^{-3}$ min^{-1}, respectively. The k value of lotus root starch was similar to that of most starches (in the order of 10^{-3} min^{-1}) [17, 27]. The k values of the granular and gelatinized starches were approximately 2 times and 5 times that of the retrograded starch, respectively, which demonstrates that the hydrolysis rates of the gelatinized and granular starches were greater than that of the retrograded starch.

Fig. 5A shows that glucose release reached maximum at 20 min, and the concentrations of peak glucose release from granular, gelatinized and retrograded starches were

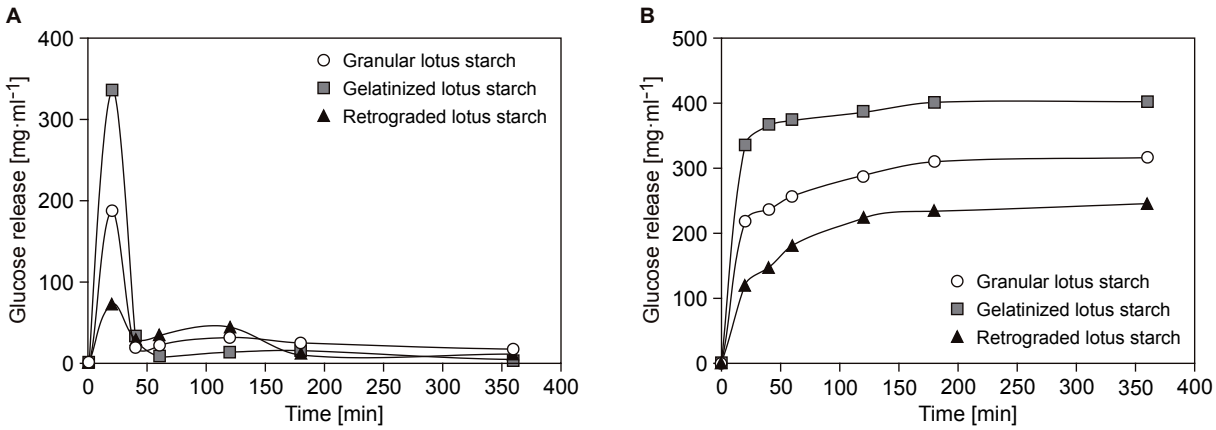

Fig. 5. Susceptibility of starch to a combination of digestive enzymes.

A – Concentration of glucose released using compound enzymes; B – concentration of glucose cumulated using compound enzymes.

Tab. 2. Contents of differently digestible fractions in starch and their glycemic and hydrolysis index.

	Granular starch	Gelatinized starch	Retrograded starch
Rapidly digestible starch at 20 min [mg·kg^{-1}]	21.45 ± 0.01	29.82 ± 0.04	16.93 ± 0.01
Slowly digestible starch at 20–120 min of incubation [mg·kg^{-1}]	63.03 ± 0.02	56.98 ± 0.01	64.32 ± 0.03
Resistant starch [mg·kg^{-1}]	15.52 ± 0.01	13.20 ± 0.02	18.75 ± 0.01
Glycemic index	66.63 ± 0.01	70.57 ± 0.02	57.83 ± 0.01
Hydrolysis index	49.03 ± 0.03	56.21 ± 0.02	33.01 ± 0.01

Data are expressed as mean ± standard deviation of triplicate measurements, means being significantly different at a 5% level.

186.45 ± 0.14 mg·l^{-1}, 335.38 ± 0.26 mg·l^{-1} and 71.47 ± 0.32 mg·l^{-1}, respectively. Fig. 5B indicates that the cumulative glucose concentrations rose drastically for the granular, gelatinized and retrograded starches before 120 min. The cumulative glucose concentrations at 120 min and 360 min were 287.39 ± 0.30 mg·l^{-1} and 316.98 ± 0.34 mg·l^{-1} for the granular starch, and 387.57 ± 0.17 mg·l^{-1} and 402.24 ± 0.26 mg·l^{-1} for the gelatinized starch, and 224.22 ± 0.35 mg·l^{-1} and 245.05 ± 0.18 mg·l^{-1} for the retrograded starch.

From Tab. 2, it can be seen the contents of RDS, SDS and RS in the granular, gelatinized and retrograded starch. Compared to the granular starch, the content of RDS increased by 39.0 % and content of SDS and RS decreased by 9.6 % and 15.0 % after gelatinization, respectively, while content of RDS decreased by 21.1 % and content of SDS and RS increased by 2.1 % and 20.8 % after retrogradation, respectively. Based on the RS content, that of the retrograded starch was the largest and that of the gelatinized starch the smallest.

GI and *HI* of the gelatinized starch were higher than *GI* and *HI* of the retrograded and granular starch. Foods are classified into three types according to *GI* value, that is, low-*GI* foods (less than 55), middle-*GI* foods (56–69) and high-*GI* foods (more than 70) [19, 28]. We can see that the granular, gelatinized and retrograded lotus root starches are middle-*GI* foods. So, it is reasonable that the lotus root starch can be a proper ingredient in developing slowly digestible starchy foods for potential health benefits by controlling the processing. Possible ways how to control the processing to obtain low-*GI* foods will be investigated in the future.

CONCLUSION

Our findings indicate that granular lotus root starch is slow digestible, as supported by the Michaelis-Menten kinetics data and hydrolysis kinetics expressed by a first-order model. Based on the large size distribution, amylose content and the *k* values of the granular starch, the granular starch contains a high amount of slowly digestible starch with moderate *GI* and *HI*. Gelatinized and retrograded starches, which are often used to prepare lotus root foods, are slowly digestible. The granular, gelatinized and retrograded lotus root starches are middle-*GI* foods. Our results provide interesting information that lotus root starch may be a low-*GI* food, which will be further verified by a human in vivo test in our future study.

Acknowledgements

This research was supported by National Natural Science Foundation of China (Grants No. 31371735 and No. 31771933) and the outstanding youth talent support program of Anhui Department of Education of China (Grant No. gxyqZD2016038).

REFERENCES

1. You, J. S. – Lee, Y. J. – Kim, K. S. – Kim, S. H. – Chang, K. J.: Ethanol extract of lotus (*Nelumbo nucifera*) root exhibits an anti-adipogenic effect in human pre-adipocytes and anti-obesity and antioxidant effects in rats fed a high-fat diet. Nutrition research, *34*, 2014, pp. 258–267. DOI: 10.1016/j.nutres.2014.01.003.
2. Geng, Z. – Zongdao, C. – Yimin, W.: Physicochemical properties of lotus (*Nelumbo nucifera* Gaertn.) and kudzu (*Pueraria hirsute* Matsum.) starches. International Journal of Food Science and Technology, *42*, 2007, pp. 1449–1455. DOI: 10.1111/j.1365-2621.2006.01363.x.
3. Zhu, F.: Structures, properties, and applications of lotus starches. Food Hydrocolloids, *63*, 2017, pp. 332–348. DOI: 10.1016/j.foodhyd.2016.08.034.
4. Sukhija, S. – Singh, S. – Riar, C. S.: Physicochemical, crystalline, morphological, pasting and thermal properties of modified lotus rhizome (*Nelumbo nucifera*) starch. Food Hydrocolloids, *60*, 2016, pp. 50–58. DOI: 10.1016/j.foodhyd.2016.03.013.

5. Guo, L. – Zhang, J. – Hu, J. – Li, X. – Du, X.: Susceptibility of glutinous rice starch to digestive enzymes. Carbohydrate Polymers, *128*, 2015, pp. 154–162. DOI: 10.1016/j.carbpol.2015.04.008.
6. Guo, L. – Hu, J. – Zhou, X. – Li, X. – Du, X.: In vitro digestibility of kudzu starch by using α-amylase and glucoamylase. Starch - Stärke, *68*, 2016, pp. 140–150. DOI: 10.1002/star.201500213.
7. Zhang, G. – Hamaker, B. R.: Slowly digestible starch: concept, mechanism, and proposed extended glycemic index. Critical Reviews in Food Science and Nutrition, *49*, 2008, pp. 852–867. DOI: 10.1080/10408390903372466.
8. Clarke, J. M. – Topping, D. L. – Christophersen, C. T. – Bird, A. R. – Lange, K. – Saunders, T. – Cobiac, L.: Butyrate esterified to starch is released in the human gastrointestinal tract. American Journal of Clinical Nutrition, *94*, 2011, pp. 1276–1283. DOI: 10.3945/ajcn.111.017228.
9. Man, J. – Cai, J. – Cai, C. – Xu, B. – Huai, H. – Wei, C.: Comparison of physicochemical properties of starches from seed and rhizome of lotus. Carbohydrate Polymers, *88*, 2012, pp. 676–683. DOI: 10.1016/j.carbpol.2012.01.016.
10. Lin, H.-M. – Chang, Y.-H. – Lin, J.-H. – Jane, J.-L. – Sheu, M.-J. – Lu, T.-J.: Heterogeneity of lotus rhizome starch granules as revealed by α-amylase degradation. Carbohydrate Polymers, *66*, 2006, pp. 528–536. DOI: 10.1016/j.carbpol.2006.04.024.
11. Naguleswaran, S. – Vasanthan, T. – Hoover, R. – Bressler, D.: Amylolysis of amylopectin and amylose isolated from wheat, triticale, corn and barley starches. Food Hydrocolloid, *35*, 2014, pp. 686–693. DOI: 10.1016/j.foodhyd.2013.08.018.
12. Chen, M.-H. – Bergman, C. J.: Method for determining the amylose content, molecular weights, and weight- and molar-based distributions of degree of polymerization of amylose and fine-structure of amylopectin. Carbohydrate Polymers, *69*, 2007, pp. 562–578. DOI: 10.1016/j.carbpol.2007.01.018.
13. Cunniff, P. A. (Ed.): Official methods of analysis of AOAC International. 16th ed. Arlington : Association of Official Analytical Chemists, 1995. ISBN: 0935584544.
14. Hermans, P. H. – Weidinger. A.: Quantitative X-ray investigations on the crystallinity of cellulose fibers. A background analysis. Journal of Applied Physics, *19*, 1948, pp. 491–506. DOI: 10.1063/1.1698162.
15. Li, X.-Y. – Wang, J. – Dong, H.-Z. – Zhang, H.-L.: Kinetic study of α-amylase in the process of starch hydrolysis by microcalorimetry. Thermochimica Acta, *579*, 2014, pp. 70–73. DOI: 10.1016/j.tca.2014.01.015.
16. Hasjim, J. – Lavau, G. C. – Gidley, M. J. – Gilbert, R. G.: In vivo and in vitro starch digestion: Are current in vitro techniques adequate? Biomacromolecules, *11*, 2010, pp. 3600–3608. DOI: 10.1021/bm101053y.
17. Al-Rabadi, G. J. S. – Gilbert, R. G. – Gidley, M. J.: Effect of particle size on kinetics of starch digestion in milled barley and sorghum grains by porcine *alpha*-amylase. Journal of Cereal Science, *50*, 2009, pp. 198–204. DOI: 10.1016/j.jcs.2009.05.001.
18. O'Brien, S. – Wang, Y./J.: Susceptibility of annealed starches to hydrolysis by α-amylase and glucoamylase. Carbohydrate Polymers, *72*, 2008, pp. 597–607. DOI: 10.1016/j.carbpol.2007.09.032.
19. Mahasukhonthachat, K. – Sopade, P. A. – Gidley, M. J.: Kinetics of starch digestion in sorghum as affected by particle size. Journal of Food Engineering, *96*, 2010, pp. 18–28. DOI: 10.1016/j.jfoodeng.2009.06.051.
20. Goñi, I. – Garcia-Alonso, A. – Saura-Calixto, F. A.: starch hydrolysis procedure to estimate glycemic index. Nutrition Research, *17*, 1997, pp. 427–437. DOI: 10.1016/S0271-5317(97)00010-9.
21. Frost, K. – Kaminski, D. – Kirwan, G. – Lascaris, E. – Shanks, R.: Crystallinity and structure of starch using wide angle X-ray scattering. Carbohydrate Polymers, *78*, 2009, pp. 543–548. DOI: 10.1016/j.carbpol.2009.05.018.
22. Cai, C. – Wei, C.: In situ observation of crystallinity disruption patterns during starch gelatinization. Carbohydrate Polymers, *92*, 2013, pp. 469–478. DOI: 10.1016/j.carbpol.2012.09.073.
23. Lindeboom, N. – Chang, P. R. – Tyler. R. T.: Analytical, biochemical and physicochemical aspects of starch granule size, with emphasis on small granule starches: A review. Starch - Stärke, *56*, 2004, pp. 89–99. DOI: 10.1002/star.200300218.
24. Tester, R. F. – Karkalas, J. – Qi, X.: Starch structure and digestibility enzyme-substrate relationship. World Spoultry Science Journal, *60*, 2004, pp. 186–195. DOI: 10.1079/WPS200312.
25. Dona, A. C. – Pages, G. – Gilbert, R. G. – Kuchel, P. W.: Digestion of starch: In vivo and in vitro kinetic models used to characterise oligosaccharide or glucose release. Carbohydrate Polymers, *80*, 2010, pp. 599–617. DOI: 10.1016/j.carbpol.2010.01.002.
26. Goesaert, H. – Bijttebier, A. – Delcour, J. A.: Hydrolysis of amylopectin by amylolytic enzymes: level of inner chain attack as an important analytical differentiation criterion. Carbohydrate Research, *345*, 2010, pp. 397–401. DOI: 10.1016/j.carres.2009.11.011.
27. Syahariza, Z. A. – Sar, S. – Hasjim, J. – Tizzottia, M. J. – Gilbert, R. G.: The importance of amylose and amylopectin fine structures for starch digestibility in cooked rice grains. Food Chemistry, *136*, 2013, pp. 742–749. DOI: 10.1016/j.foodchem.2012.08.053.

Received 2 August 2017; accepted 6 October 2017; published online 6 December 2017.