Preparation, structural characteristics and digestibility of resistant starches from highland barley, oats and buckwheat starches

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Summary

The objective of this study was to investigate the structural characteristics and digestibility of resistant starches from highland barley starch, oats starch and buckwheat starch. The three cereal starches were subjected to enzymatic hydrolysis (thermostable α-amylase 3 $U \cdot g^{-1}$, pullulanase 40 $U \cdot g^{-1}$), autoclaved (121 °C, 30 min), stored under refrigeration (4 °C, 24 h) and dried (40 °C, 12 h). Particle size distribution analysis showed that the distribution of resistant starches was more concentrated than those of the native starches. Oat resistant starches had smaller granules and average grain diameter. Scanning electron micrographs of resistant starches presented an irregular shape that was different from native starches. The crystallinity pattern of three cereal resistant starches revealed B-type according to X-ray diffractograms. In vitro digestibility of three samples was analysed using a multi-enzyme dialysis system, which showed that the glycaemic index (*GI*) values of highland barley resistant starches ($GI = 52$), oat resistant starches ($GI = 48$) and buckwheat resistant starches ($GI = 45$) were significantly lower than those of native starches.

Keywords

resistant starch; highland barley; oats; buckwheat; structure; digestibility

Diet customs of people have been changed significantly in recent years with the growing demand for functional foods. Resistant starch (RS) of grains including buckwheat, highland barley and oats has become very attractive for researchers due to its functional properties [1]. RS is a minor fraction of starch that escapes digestion in the human small intestine and may be fermented in the colon with production of metabolically active short chain fatty acids (SCFAs) [2]. RS has been classified into five general subtypes called RS1–RS5. The five distinct classes of RS in foods are: RS1 – physically inaccessible starch; RS2 – types of raw starch granules as in raw banana and potato; RS3 – retrograded or crystalline non-granular starch, an indigestible starch fraction found in cooled or cooked starchy foods, thermal stability of which enables its existence after most normal cooking operations and use in a wide variety of foods; RS4 – chemically modified starch and RS5 – amylose-lipid complex [3–6].

The functional properties of RS that positively influence the digestive tract include enhanced fermentation and laxation, increased uptake of minerals such as calcium, as well as increase in probiotics [7]. From a nutritional point of view, RS is important for human health to maintain the level of blood glucose and assists in the control of diabetes [8, 9]. Our recent in vivo study indicated that sorghum RS helps the body to prevent and treat obesity through mechanisms including synthesis and secretion of leptin and adiponectin, and improvement in intestinal flora [10]. RS from different ingredients are now commercially available in the European market to be incorporated in food formulations to increase RS or fibre content [11].

Many investigators studied cereal starches and found that buckwheat, highland barley and oats starches have higher contents of slowly digested starch as well as lower glycaemic index (*GI*) compared with rice and wheat starch [12–14]. The

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three grains therefore can be a potential source of RS with unique properties. RS is now mainly prepared from rice and maize starches [15–17]. However, there are seldom researches on different properties of RS prepared from various kinds of cereal materials, and RS digestibility and glycaemic response for the majority of coarse grains is poorly characterized in current literature. The glycaemic response has been related to the rate of digestion and absorption of starchy foods with help of in vitro experiments, which are mimicking in vivo digestion processes. In vitro procedures enable to predict in vivo conditions reasonably, and the various procedures for in vitro studies were discussed previously [18–20].

The objective of the present study was to prepare RS3 from isolated starches of highland barley, oats and buckwheat to characterize their physico-chemical properties and digestibility, aiming at offering a theoretical basis for the application of resistant starch in various kinds of food.

MATERIALS AND METHODS

Materials

Highland barley, oats and buckwheat were obtained from Tibet Academy of Agriculture and Animal Husbandry Sciences (Xizang, China), Hebei Kangxi Oats Food (Hebei, China) and Tpftz Aixin Foodstuffs (Tianjin, China), respectively. Whole seeds of highland barley, oats and buckwheat were ground in a cyclone mill to pass through a sieve with 0.30 mm aperture. The whole seed flours were partially defatted with hexane (0.1 $g \cdot ml^{-1}$) for 3 h, and allowed to air dry overnight.

Cellulase (EC 3.2.1.21), protease (EC 3.4.21.14), thermostable α-amylase (EC 3.2.1.1), pullulanase (EC 3.2.1.41), porcine pancreatic α-amylase (EC 3.2.1.1) and pepsin (EC 3.4.1) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Amyloglucosidase (EC 3.2.1.3) and resistant starch assay kit were purchased from Megazyme International (Wicklow, Ireland). All other chemicals used were of analytical grade.

Isolation of highland barley, oats and buckwheat starches

A procedure for the isolation of starches was developed based on the enzymatic method [21] with modification. Defatted sample was rinsed and homogenized in a blender with distilled water for 2 min at maximum speed. The starch slurry was passed through a sieve with 0.15 mm aperture. The sieve-through was centrifuged (3050 ×*g*, 15 min). The sediment in distilled water (0.1 $g \cdot ml^{-1}$) was added cellulase (50 $U \cdot g^{-1}$ defatted sample) and incubated at 45 °C with constant stirring for 3 h. Then the suspension was centrifuged (3050 ×*g*, 10 min), the supernatant was decanted and the layer on top of the white starch sediment was removed. The obtained sediment suspended in distilled water (0.2 $g \cdot ml^{-1}$) was added protease (100 $U \cdot g^{-1}$ defatted sample) and incubated at 45 °C with constant stirring for 30 min. The suspension was centrifuged (3050 ×*g*, 10 min). The white starch sediment was collected and washed with distilled water three times and freeze-dried. The obtained starches were named HS for highland barley starch, OS for oats starch and BS for buckwheat starch.

Preparation of resistant starches

The highland barley, oats and buckwheat RS were obtained according to the method of Sievert and Zhou [22, 23] with some modification. The starch slurry (250 $g \cdot l^{-1}$, pH adjusted to pH 6.0 with dilute HCl) was incubated in a thermostatic water bath at 100 °C for 30 min. Then, the temperature was adjusted to 85 °C and the solution was treated with thermostable α-amylase (3 $U \cdot g^{-1}$ dry starch, pH 5.4) for 30 min. After that, the solution was added pullulanase (40 $U \cdot g^{-1}$ dry starch, pH 4.5) and incubated in a water bath at 55 °C for 8 h. The hydrolysed solution was then autoclaved at 121 °C for 30 min, cooled to room temperature and stored at 4 °C for 24 h. Finally, after being centrifuged (3050 ×*g*, 20 min), the sediment was collected, washed and dried in an oven at 40 °C for 12 h. The obtained resistant starches were named HRS for highland barley RS, ORS for oats RS and BRS for buckwheat RS. All the samples were ground and screened through a sieve with 0.18 mm aperture.

Chemical composition

The moisture content was analysed by oven drying method with reference to AOAC methods 934.01/4.1.03 [24]. The protein and lipid contents were assessed according to AOAC methods 988.05/4.2.03 [25] and 920.39/4.5.01 [26]. Resistant starch was determined using the Megazyme RS assay kit (Megazyme International), which is based on removal of non-resistant starch with α-amylase and amyloglucosidase. All samples were analysed in triplicate.

Apparent amylose content

Apparent amylose content (AAM) was determined according to the iodine binding-based method [27].

Particle size distribution analysis

An amount of 0.1 g of sample was mixed with 10 ml of distilled water and fully dispersed by ultrasonic treatment. The particle size distribution of the sample was analysed by a particle size analyzer (Winner3001; Jinan Micro-Nano Particle Technology, Jinan, China).

The approach to define the distribution width was to cite three values on the x-axis, namely, D10, D50, and D95. D50, median, is defined as the diameter where half of the population lies below this value. Similarly, 95 percent of the population lies below D95, and 10 percent of the population lies below D10.

Scanning electron microscopy

The samples were uniformly dispersed on double-sided Scotch tape (Jeol, Tokyo, Japan), fixed in a sample holder, and coated with a layer of gold using ion-sputtering instrument. Samples were observed under a scanning electron microscope (JSM-6490LV; Jeol, Tokyo, Japan) at 10 kV.

X-ray diffraction

The crystal structure was examined by X-ray diffractometer (XPert Powder; PANalytical, Almelo, Netherlands) to obtain the diffraction patterns with the following conditions: Cu-Kα radiation with voltage 40 kV and current 20 mA, diffraction angle (2θ): 60°–5° and scanning step of 0.02°.

In vitro digestibility

Sample digestibility was measured by a rapid in vitro digestibility assay based on glucometry [18] with some modifications. A volume of 0.5 ml of ground sample solution (1%, w/w) was treated with 10 ml simulated gastric fluid containing pepsin (pH 2.0) and 6 ml phosphate buffer (pH 6.8). The mixture was incubated at 37 °C for 30 min in a reciprocal water bath (1.4 Hz). The pH value of the solution was continuously maintained at 1.2 ± 0.1 by the addition of 0.2 mol·l^{-1} HCl. Then, the pH value was changed to 6.0 ± 0.1 by the addition of 0.2 mol·l^{-1} NaOH. Volumes of 10 ml of phosphate buffer and 8 ml of simulated intestinal fluid containing porcine pancreatic α-amylase (290 U·ml^{-1}) and amyloglucosidase (15 U·ml^{-1}) were added, and pH was adjusted to 6.8 ± 0.1 by the addition of 0.2 mol·l^{-1} NaOH. The solution was placed in a dialysis bag with molecular weight cutoff 14000 Da. Incubation in the water bath (37 °C, 1.4 Hz) proceeded for 180 min. During the simulated small intestine digestion process, supernatants (0.5 ml) were collected to analyse glucose at specific periods (0, 5, 10, 20, 30, 60, 90, 120 and 180 min) by 3,5-dinitrosalicylic acid colorimetry [28]. The rate of starch digestion was represented as the percentage of total starch hydrolysed at specific periods. The starch amount was calculated by multiplying glucose levels by 0.9.

The kinetics of starch digestion were studied by the method of Goñi [29]. The areas under hydrolysis rate curves (*AUC*, 0–180 min) were calculated, using the equation given below, for all products. Hydrolysis curves for each product follow a first order equation (Eq. 1):

$$C = C_\infty (1 - \exp(-kt)) \quad (1)$$

where C is the concentration at t time, C_∞ is the equilibrium concentration, k is the kinetic constant and t is the chosen time.

AUC was calculated as the integral of the kinetic equation. The hydrolysis index (*HI*) of each sample was calculated by dividing *AUC* (at a specific time period) by *AUC* of a fresh white bread [30]. The predicted *GI* was estimated using Eq. 2 with a correlation coefficient $r = 0.89$, $P < 0.05$ [31]:

$$GI = 39.71 + 0.549\, HI \quad (2)$$

where value 39.71 and value 0.549 represent the intercept and slope of the linear equations, respectively.

Statistical analysis

The differences between the mean values of multiple groups were analysed by one-way analysis of variance (ANOVA) using Duncan's multiple range tests. *P* values lower than 0.05 were considered significant. Software SPSS 16.0 (IBM, Armonk, New York, USA), Microsoft Excel (Microsoft, Redmond, Washington, USA) and Origin 8.6 (OriginLab Guangzhou Office, Guangzhou, China) were used to analyse and report the data.

RESULTS AND DISCUSSION

Chemical composition

Tab. 1 shows the chemical composition of the three cereal starches and their RS samples. Moisture ranged from 74.1 g·kg^{-1} to 88.7 g·kg^{-1}, protein content was 2.1 g·kg^{-1} to 5.5 g·kg^{-1}, and lipid content was 1.2 g·kg^{-1} to 4.7 g·kg^{-1}. Compared with HRS and BRS, ORS had the highest content of amylose (884.2 g·kg^{-1}) and RS (850.9 g·kg^{-1}).

Amylose can form complexes with various organic or inorganic ligands [32, 33]. Chandrashekar [34] found that protein plays an embedding effect on starch granule, and starch granule proceeds anabiosis after the removal of this protein. In addition, the foreign protein can also in-

Tab. 1. Contents of basic components in cereal starches and resistant starches.

Material	Moisture [g·kg^{-1}]	Protein [g·kg^{-1}]	Lipids [g·kg^{-1}]	Amylose [g·kg^{-1}]	Resistant starch [g·kg^{-1}]
Highland barley starch	86.9 ± 0.6^{b}	33.8 ± 0.3^{a}	16.3 ± 0.8^{b}	109.7 ± 0.6^{c}	53.8 ± 0.5^{a}
Oats starch	86.7 ± 0.6^{b}	20.1 ± 0.4^{c}	18.2 ± 0.5^{a}	210.4 ± 0.7^{a}	30.8 ± 0.5^{c}
Buckwheat starch	89.8 ± 0.5^{a}	31.7 ± 0.3^{b}	15.5 ± 0.7^{b}	165.3 ± 0.8^{b}	40.2 ± 0.4^{b}
Highland barley resistant starch	88.7 ± 0.5^{a}	5.5 ± 0.4^{a}	4.7 ± 0.7^{a}	830.3 ± 0.2^{c}	784.0 ± 0.4^{c}
Oats resistant starch	74.1 ± 0.7^{b}	2.1 ± 0.2^{b}	1.2 ± 0.6^{c}	884.2 ± 0.3^{a}	850.9 ± 0.5^{a}
Buckwheat resistant starch	86.8 ± 0.4^{a}	3.8 ± 0.3^{a}	3.4 ± 0.5^{b}	851.1 ± 0.2^{b}	814.0 ± 0.6^{b}

Values represent the means of triplicates. Values with the same superscript in a column did not differ significantly ($P < 0.05$).

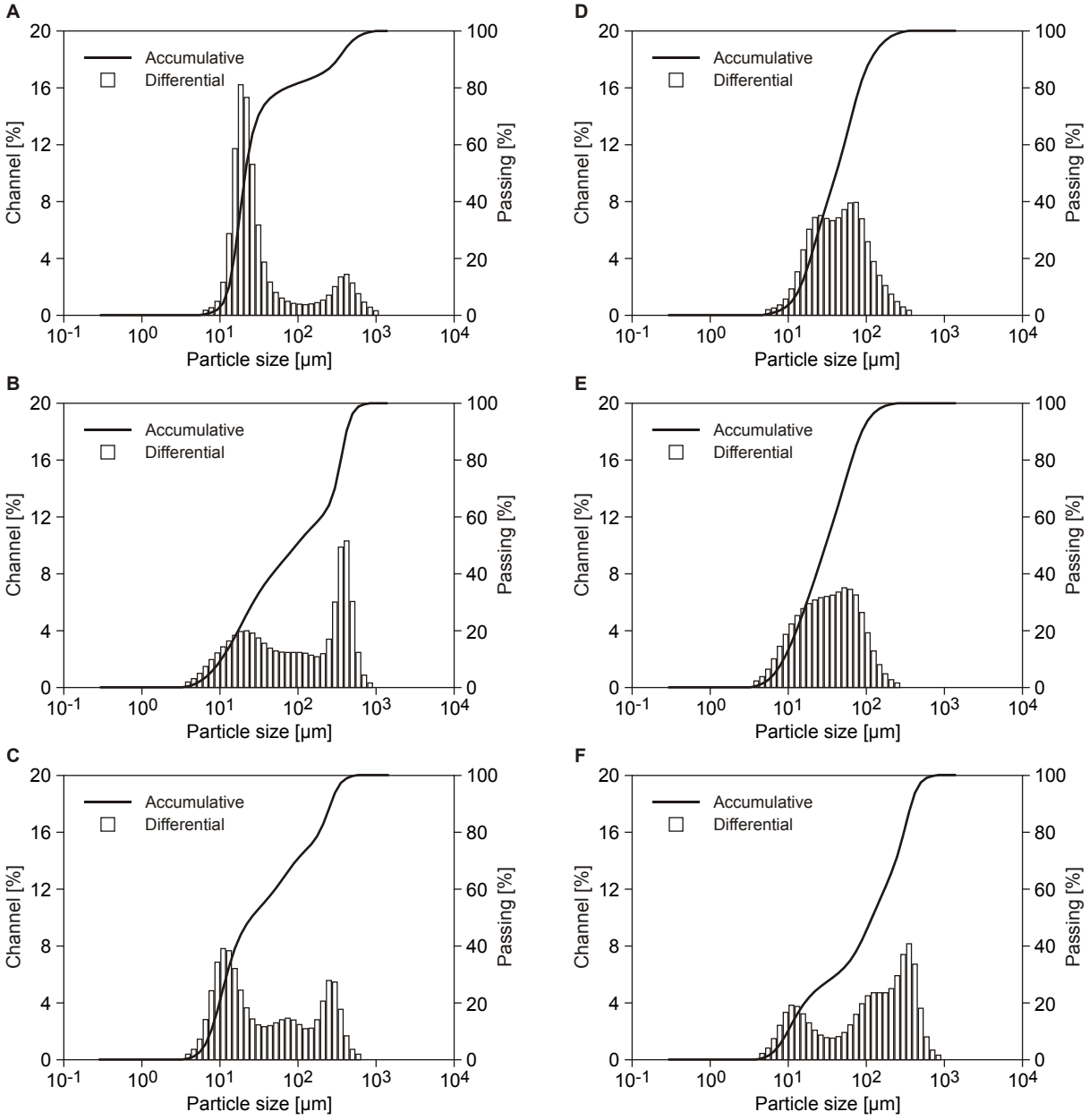

Fig. 1. Particle size distribution of cereal starches and resistant starches.

A – highland barley starch; B – oats starch; C – buckwheat starch; D – highland barley resistant starch; E – oats resistant starch; F – buckwheat resistant starch.

teract with amylose molecule by hydrogen bond to bind the starch molecules, which inhibits retrogradation of amylose and reduces the content of RS in the food. Using a heat-treatment method that affects the formation of resistant starch of wheat, KING and TAN [35] found that resistant starch could be produced from low amylose starches and botanical sources through enzymatic hydrolysis. This experiment used enzymatic hydrolysis to remove lipids, protein and fibre during the isolation process of starch, which increased the formation of amylose double helix as much as possible, and pullulanase was used to debranch the purified starch and then to obtain a higher content of resistant starch.

Particle size distribution

Particle size distribution of three cereal starches and RS is displayed in Fig. 1. The curve on Fig. 1 represents the accumulative distribution of particle sizes while the strip means the differential distribution. It can be seen that D95 values of cereal starches (313–700 μm) were greater than those of RS (111–438 μm), and the differen-

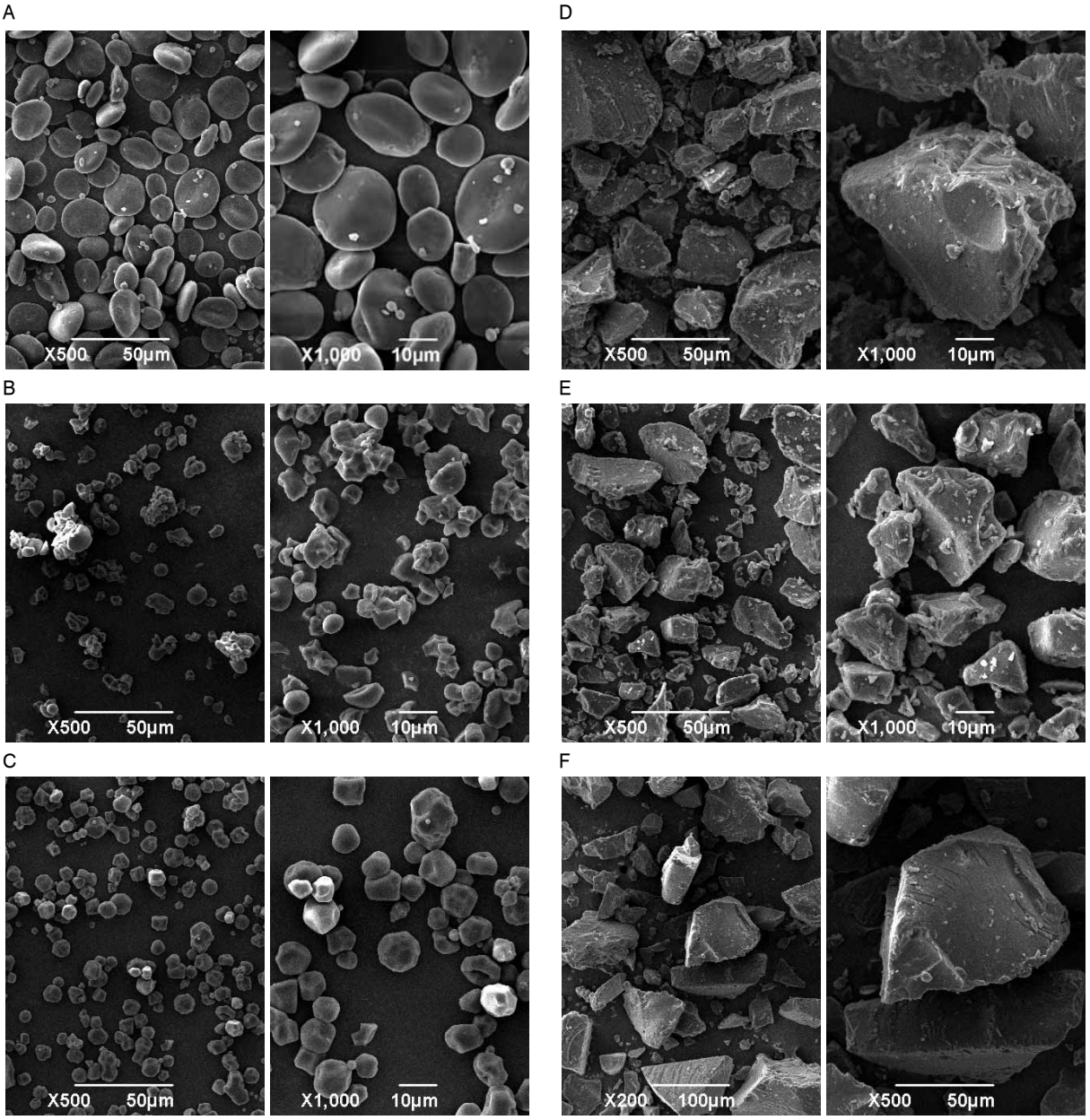

Fig. 2. Scanning electron micrographs of cereal starches and resistant starches.

A – highland barley starch; B – oats starch; C – buckwheat starch; D – highland barley resistant starch; E – oats resistant starch; F – buckwheat resistant starch.

tial distribution of cereal starches displayed two peaks, while that of RS displayed a single peak, except for BRS. This means that the particles in RS were more concentrated than in starches. The D10 and D50 values of ORS were 8.79 μm and 30.18 μm, respectively, which were both smaller than those of HRS (14.36 μm, 41.22 μm) and BRS (9.92 μm, 119.3 μm). Previous study [36] reported that there was a positive correlation between amylose content and the quantity of small granules, and the average grain diameter of granule correlated negatively with amylose content. The higher was amylose content, the smaller was the average diameter of granule. In pixels diameter (Fig. 1) of three cereal RS, ORS had the most of small granules and the smallest average grain diameter, which means containing the highest amount of amylose. Besides that, resistant starch was mostly made up of amylose, which is in line with the result that ORS had the highest resistant starch content. The overall granule diameter of RS was greater than that of starch, which might be because of starch gelatinization and the polymerization process [37].

Morphological characteristics

Scanning electron micrographs of the granules of three cereal starches and RS are shown in Fig. 2. The three cereal starch granules presented different morphologies according to Fig. 2. Similar to rice starch particles, BS granules are also polygon-shaped. Besides that, BS granules were spherical, concave and had a plurality of edges. HS granules were oval or spherical with smooth surface. OS granules, which are similar to those of durian seeds [38], were complex particles with no fixed form, with different degrees of sharp edges and corners. However, three cereal RS granules lost the granule structure of their native starch and formed an irregular shape without a significant difference.

The cause of resistant starch formation is the aging of amylose. During aging, molecules of curly amylose get closer to each other gradually, and form a double helix through intermolecular hydrogen bonds. Double helices overlap each other, and then form small crystal nucleus, which continuously grows, ripens until a bigger amylose crystal is formed [39]. This could be regarded as the formation of irregular granule structure in scanning electron microscopy picture. Meanwhile, the irregular granule structure of RS contributes to the reduction in the activity of α-amylase [40]. The amylose crystals could prevent the active site of amylase from getting close to the α-1,4 glycoside bond in the crystal, which also explains the antidiastase activity of amylose crystals [41].

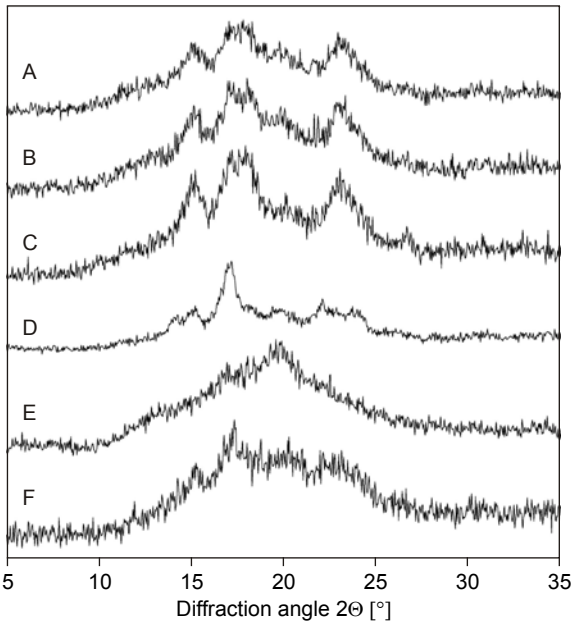

Fig. 3. X-ray diffractograms of cereal starches and resistant starches.

A – highland barley starch; B – oats starch; C – buckwheat starch; D – highland barley resistant starch; E – oats resistant starch; F – buckwheat resistant starch.

X-ray diffraction

Starch granules in the crystalline alignment give rise to the peaks in X-ray diffractograms (XRD), whereas starch granules in amorphous regions contribute to the diffuse regions of XRD patterns. Crystal structure of starch can be classified into A, B, C and V type. Among these four types, C type is the combination of A and B type [42]. A type starch crystallinity is observed mainly in grain starches, such as wheat and rice starch, with strong reflections at 15°, 17°, 18° and 23°. B type crystallinity is observed mainly in starch tubers, fruits such as potato and banana starch, with strong reflections at 5.6°, 17°, 22° and 24°. V type crystallinity is generally related to amylase-lipid complex, with strong reflections at 14.5° and 19.5° [43, 44].

The crystallinity pattern of three cereal starches (HS, OS, BS) and RS (HRS, ORS, BRS) are displayed in Fig. 3. By comparison, it can be clearly seen that there was no significant difference among the crystal structure of three cereal starches in this experiment. Three starches had a strong diffraction peak at diffraction angles (2θ) 15°, 17°, 18° and 23°, so all crystal patterns were A type. However, there were big changes in diffraction peaks of RS. HRS and BS had strong dif-

fraction peaks at 17°, 22° and 24°, which manifested B type crystal pattern. ORS had a strong peak at 19.88° (2θ), and had weak diffraction peaks at 7.5°, 13° and 31°, which could mean that V type structure appeared in it. As has been reported previously, hydrothermal treatment can change the crystal structure pattern from A to B [45]. In this study, α-amylase and pullulanase were used to prepare cereal RS, the two enzymes co-hydrolysing the starch chain to a certain straight-chain length, and then the straight chain formed a double helix in the retrogradation process. During the retrogradation process at a low temperature (e.g. 4 °C), B type crystal pattern was formed [23]. The crystal patterns of RS were mainly the B and V type, as could be concluded from X-ray diffractograms. The similar pattern was also observed in sago RS [46] and maize RS [47].

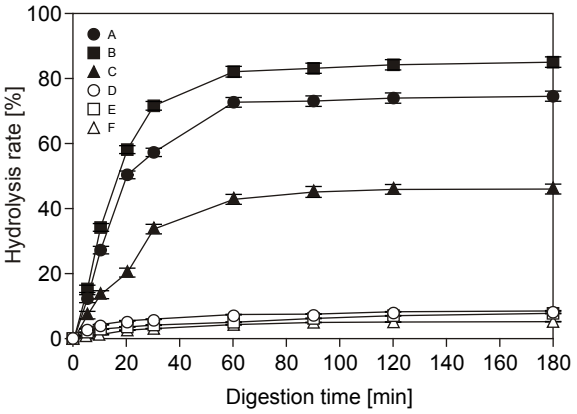

Fig. 4. Hydrolysis rate of cereal starches and resistant starches.

A – highland barley starch; B – oats starch; C – buckwheat starch; D – highland barley resistant starch; E – oats resistant starch; F – buckwheat resistant starch.

In vitro digestibility

Digestion kinetics analysis help us better understand the digestibility of substances and physiological characteristics of the gastrointestinal tract, thereby effectively evaluating the nutritional value of different foods. As shown in Fig. 4, the hydrolysis rates of three starches were different during the first 30 min. Hydrolysis rate of BS ranged from 10% to 30%, which was significantly lower than OS (30–70%) and HS (20–60%) ($P < 0.05$). Hydrolysis rate of HRS was greater than ORS and BRS ($P > 0.05$). Starches from different sources with different morphological characteristics and structures had a different rheology, which affected the hydrolysis rate of starch [48].

Up to 60 min, hydrolysis rate of the three starches increased rapidly. That of OS increased even to about 80%, which might have been due to the molecular structure of the starch granules, which was loose and relatively easy to swell. After 90 min of hydrolysis, digestibility of the three starches gradually reached a plateau, indicating that there was a certain amount of amylose starch. However, at this time, hydrolysis rate of RS were still significantly lower than that of native starches ($P < 0.05$), in which the largest hydrolysis rate of RS was only about 10%, indicating that digestibility of RS was low. The indigestibility of RS is known to be due to recrystallization, when moist gelatinized starch is stored for a prolonged period of time [49]. High levels of RS in foods could increase the quality of chyme, promote proliferation of beneficial bacteria and increase the production of short-chain fatty acids in the colon [50].

Amylose content of ORS was 93.4%, which was higher than in BRS and HRS. However, ORS did not show a lower hydrolysis rate regarding starch digestibility. This might have been because amylose content was just one of the factors affecting starch digestibility. Several other factors may play a role in this process and have an inhibitory effect on starch degradation, reducing the total starch digestibility [51]. Our results are in agreement with those of SVIHUS et al. [52], who found that, compared with the large starch granules, small particles had a greater starch digestibility. Formation of lipid complexes also has an effect on digestive enzymes and affects the utilization of nutrients [53].

The kinetic parameters are shown in Tab. 2. The equilibrium concentration (C_∞) of highland barley RS, oats RS and buckwheat RS were 91.61 mmol·l^{-1}, 86.54 mmol·l^{-1} and 88.61 mmol·l^{-1}, respectively, which was significantly less than the equilibrium concentration of native starch ($P < 0.05$). This may be due to the high resistant starch amylose content prone to retrograde, resulting in reduction of the equilibrium concentration of RS. Meanwhile, k values of three cereal RS were significantly lower than those of native starches ($P < 0.05$), which indicated that RS reached equilibrium concentration faster than native starches. In addition, compared with BRS and HRS, ORS had the lowest k value (0.017 min^{-1}) and the lowest equilibrium concentration (86.54 mmol·l^{-1}). This may be due to its high content of amylose starch. Tab. 2 shows that cereal starch had higher *HI* than RS. BRS had the lowest *HI* (10.18) compared with ORS and HRS.

Hydrolysis of starch is a key factor for controlling *GI*. In vitro hydrolysis of starch could be used to evaluate the GI values, which had a high cor-

Tab. 2. In vitro digestion parameters.

Sample	C_∞ [mmol·l^{-1}]	k [min^{-1}]	AUC [mmol·l^{-1}·min^{-1}]	Hydrolysis index	Glycaemic index
Highland barley starch	95.03 ± 0.02^{a}	0.033 ± 0.011^{b}	4334.61 ± 0.11^{b}	36.35 ± 0.01^{b}	59.67 ± 0.02^{b}
Oats starch	95.69 ± 0.02^{a}	0.073 ± 0.011^{a}	6035.24 ± 0.02^{a}	50.61 ± 0.02^{a}	67.49 ± 0.02^{a}
Buckwheat starch	93.83 ± 0.02^{b}	0.027 ± 0.014^{c}	3095.98 ± 0.06^{c}	25.96 ± 0.02^{c}	53.96 ± 0.02^{c}
Highland barley resistant starch	91.61 ± 0.01^{c}	0.026 ± 0.009^{c}	2592.23 ± 0.41^{d}	21.74 ± 0.01^{d}	51.65 ± 0.02^{d}
Oats resistant starch	86.54 ± 0.02^{e}	0.017 ± 0.012^{d}	1878.24 ± 0.16^{e}	15.75 ± 0.02^{e}	48.36 ± 0.02^{e}
Buckwheat resistant starch	88.61 ± 0.01^{d}	0.021 ± 0.010^{c}	1213.93 ± 0.21^{f}	10.18 ± 0.02^{f}	45.30 ± 0.01^{f}

Values represent the means of triplicates. Values with the same superscripts in a column did not differ significantly ($P < 0.05$). C_∞ – equilibrium concentration, k – kinetic constant, AUC – area under the hydrolysis curve.

relation with blood glucose concentration [54]. Starch was easily digested and the released glucose quickly caused postprandial glucose substantial changes, which is very unfavorable to diabetic patients. However, *GI* values of three RS ranged from 40 to 51, which were significantly lower than those of native starch ($P < 0.05$). The results indicate that RS could help to control postprandial hyperglycaemia and avoid the sharp rise and fall of blood glucose regarding diabetes. Although high-*GI* food would cause more insulin secretion, it would also increase food intake [55]. Among the materials selected in this experiment, ORS had the high amylose content and relatively good digestion kinetics. Therefore, it can be preferred as a food ingredient regarding diabetes.

CONCLUSION

Resistant starch could be prepared from highland barley, oats and buckwheat starch by hydrolysis of non-resisitant starch with thermostable α-amylase and pullulanase, followed by heating aqueous starch isolated in autoclave and cooling. The dual-enzymes modification not only changed the microstructure of three cereal starches but also reduced their digestibility. These findings would help to promote the discovery and development processes for designing novel cereal foods to manipulate postprandial glycaemic response. The research on the relationship between the structure and properties of these RS, and on their fermentation in the colon, is in progress.

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