Starch Properties and Structure of A Wheat Mutant High in Resistant Starch

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Abstract: Starch properties and structure of a wheat mutant (WRS-1) high in resistant starch (RS) was compared to that of the wild type (Sumai No.6). In cooked hot flour and retrograded flour, the amounts of RS in mutant WRS-1 were 7.8 and 9.4 times of wild type (0.5 in hot flour and 0.6 in retrograded flour), respectively, and correspondingly the slower and incomplete starch hydrolysis was also in both types of samples. WRS-1 had a higher λmax of absorbance, blue value (BV), apparent amylose content (AAC), and contained the increased intermediate and long chains with 18≤ degree of polymerization (DP) and decreased short chains with DP≤17. Two types of starch granules, large A in lenticular shape and small B in spherical shape, were observed in both mutant and wild types. However, the starch granules in WRS-1 were relatively larger and some were irregularly-shaped. High content of total starch (TS), decreased contents of lipid (LC) and protein (PC), lower onset temperature (T_o), peak temperature (T_p), final temperature (T_c), and the enthalpy of gelatinization (ΔH_{gel}) were observed in WRS-1.

Keywords: Wheat, resistant starch, starch properties, starch structure.

INTRODUCTION

Starch is the primary source of dietary carbohydrate and energy intake [1-2]. In a view of starch digestion, common starch contains a higher percentage of digestible fraction (digestible starch, DS) and a lower percentage of the non-digestible fraction called as enzymatic resistant starch (RS) in the major staple starchy crops such as rice and wheat [3-5]. RS is defined as 'the sum of starch and the products of starch degradation not absorbed in the small intestine of healthy individuals' [6]. The slowly digested RS results in decreased postprandial glucose and insulin responses, which is beneficial for a person with diabetes and hyperlipidemia [7-12]. The RS fermented in the colon may produce many short-chain fatty acids (SCFA) that are helpful in preventing colonic diseases [12-16].

Wheat is a major cereal crop that is used as a food ingredient in bread, noodles and many bakery products [17]. The RS contents were very low with 0.08% in the native wheat flour [18], 0.3%~1.0% in wheat flour bread and white bread [19], and 0.54%~1.03% in wheat-based *poori* by different treatments [20]. A novel high-amylose barley cultivar (*Hordeum vulgare* var. *Himalaya 292*) with altered starch synthesis and less total starch but more amylose and RS were found to lower plasma cholesterol and alter the indices of large-bowel fermentation in pigs [14]. High-amylose wheat generated by RNA interference was reported to improve the indices of large-bowel health in rats [21]. These results indicate that high-amylose barley and wheat have significant potential to improve human health through its resistant starch content. However, high-amylose grains are poor in eating quality and the starch is generally used as an additive for the food industries in most cases. To increase the RS in bread, high-amylose wheat/maize flour or wheat bran was used as an additive in bread making [22-24], or the normal starch was physically or chemically modified before being processed [25]. However, carbohydrate consumption from white bread is five times that of the whole wheat [26], which has benefits by reducing the glucose, insulin and blood lipids [27].

In the context of globally increasing levels of obesity and diabetes [28], breeding for staple food crops such as wheat, rice and other starchy crops with high RS content and better quality is of particular interest, as they have great impacts on the dietary prevention of diabetes and hyperlipidemia [5]. High-RS mutant rice was identified in our previous studies [5, 29]. A simplified methodology suitable for screening the starchy crops high in RS indirectly was established, and a high-RS wheat mutant (WRS-1) with intermediate content of amylose was isolated from a commercial variety 'Sumai No.6' in Huihe River valley of China. In the current paper, starch properties and structure of this mutant were comparatively studied with the wild type, which will provide information for plant breeders to improve RS in wheat and for food technologists to develop high-RS wheat-based foodstuffs.

MATERIALS AND METHODOLOGY

Materials

Wheat mutant 'WRS-1' high in resistant starch was identified from the commercial wheat variety 'Sumai No.6' by induced mutation. Briefly, the dry seeds with 13.5% seed moisture were irradiated by 300 Gy Cobalt-60 gamma rays. The resulting irradiated seeds were directly sown in the field and at maturity seeds were bulkily harvested from each M_1

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plant. The M_2 seeds were sown on a seedling bed and then seedlings were individually transplanted into the field at the 5^{th}-6^{th} leaf-age stage. At maturity seeds were individually harvested for preliminary screening plants high in resistant starch. The isolated candidates were grown into M_3 plant lines for further verification of real mutations. In the subsequent three generations at $M_{4\text{-}5\text{-}6}$, the stabilities of identified mutant mutation high in resistant starch were evaluated among environments. One of mutant labeled as WRS-1 at M_7 generation was used for the current study. 'Sumai No.6' is widely planted in the Huihe River Valley of China.

Sample Preparation

Wheat grains were dehulled by hand and then ground to whole-meal flour using a Quadrumat Jr. Mill (Brabender, Duisburg, Germany). The resulting flour was sieved with a 100-mesh for apparent amylose content (AAC), crude lipid content (LC), protein content (PC), DSC thermal properties, total starch (TS) and resistant starch (RS) analysis. Starch was isolated by a NaOH method described by Verwimp, Vandepytte and Marrant [30]. The isolated starches were dried on an oven at 40°C for 24 h and the resulting starches were gently sieved with 100-mesh and stored in a sealed plastic bag for starch morphology scanning. According to practical applications in daily life and food industries, freshly hot processed and retrograded samples were chosen for this analysis. The freshly hot cooked flour were prepared with the ratio of water to flour 4:1 and cooked 20 mins in boiling water by a Cooker and then kept in warm-holding status (at 50°C) 10 mins. The freshly cooked flour was kept under room temperature for 24 h as retrograded samples.

Apparent Amylose, Total Starch, Resistant Starch, Lipid and Protein Determination

AAC was determined according to the method based on the amylose-iodine blue value at λ=620 [31]. LC was determined by the AACC method [32] and PC was measured with the microkjeldahle method [31]. The contents of RS in the hot and retrograded samples were determined by a Megazyme RS Kit (Megazyme International Ireland Ltd., Co. Wicklow, Ireland). TS was determined according to the protocol described by Garcia-Alonso *et al*. [33].

In Vitro Kinetics of Starch Digestion

In vitro starch hydrolysis was determined according to Goñi *et al.* [34] with minor modifications. After incubating with 300 IU pepsin (Amresco) for 1 h, starch hydrolysis was initiated by adding 3.0 IU porcine pancreatic alpha-amylase (Megazyme) in Na-K phosphate buffer. The final volume was 30 ml. The reaction mixture was incubated at 37°C with moderate agitation. Two aliquot of 0.5 ml solution were taken from each flask every 30 min from 0 to 3 h and the alpha-amylase inactivated by adding 0.5 ml 1.2 M glacial acetic acid. The total reducing sugar was determined by the DNS regent (3,5-dinitrosalicylic acid). The extent of hydrolysis was calculated as the percentage (%, maltose equivalent) of starch degraded to maltose in total starch. Each sample was analyzed in triplicate.

Differential Scanning Calorimetry (DSC)

The thermal properties of flour isolated from wheat were determined using a differential scanning calorimeter (DSC) (Q100T, TA Inc., Newcastle, DE) and calculated with the Universal Analysis program, version 3.8B. Five-milligram samples were placed in an aluminum cup, and 20 μL of distilled water was added. The cup was hermetically sealed and then heated from 30°C to 110°C at a rate of 10°C/min. The major parameters of the DSC profile were described as onset temperature (*T*O), peak temperature (*T*P), enthalpy of gelatinization (*ΔH*gel), and final temperature (*T*C).

Scanning Electron Microscope

Starch powders were homogeneously stuck on double-adhesive tape fixed on a metallic stub, then treated in an IB-5 Ion Coater (Eiko Co., Japan) for 30 min under argon atmosphere, coated with the Pt ion, and visualized with a scanning electron microscope (XL30ESEM, Philips Co., Holland) at 20 kV. Micrographs of starch samples were taken at 500×magnification, and the sizes of starch granules in three of randomly selected scopes were measured in the Adobe Photoshop Elements 2.0.

X-Ray Diffraction Pattern

X-ray diffraction patterns of the wheat flour were measured with copper Kα radiation (λ=0.154 nm) using a diffractometer (D/max 2550PC, Rigku Inc., Japan) equipped with the Universal Analysis software, Version 3.8B. The diffraction was operated at 30 mA and 40 kV. The region of two-theta angle (2θ) was scanned over the range from 3.0 to 70.0° with a 0.05°of step size and a count time of 2 s. The estimated cystallinity was analyzed by assessing the contribution of amorphous features to the total diffraction intensity over the angular range 5-35° 2θ with the SigmaPlot software (SYSTAT, USA).

Chain-Length of Amylopectin

Milled wheat flour was debranched as described by Umemoto *et al*. [35] and the amylopectin chain-length (ACL) distribution profile determined by high performance anion exchange chromatography with pulsed amperometric detection (HPAE-PAD) [36].

Statistical Methods

The data obtained were subjected to one way analysis of variance followed by Duncan's multiple-range tests.

RESULTS AND DISCUSSIONS

Resistant Starch and *In Vitro* Hydrolysis

RS and glycemic index (GI) are two indicators important for starch digestibility. In numerous studies, RS is used as a predictor of slow release of glucose and GI is predicted by *in vitro* model of starch hydrolysis [34, 37-38].

The RS contents in WRS-1were 3.82% in hot wheat flour and 5.36% in retrograded flour (Table **1**). The amounts of RS were significantly increased as 7.8 and 9.4 times of that of wild type (0.5% in hot wheat flour and 0.6% in retrograded flour) respectively. The extent of RS enrichment caused by starch retrogradation was also different between mutant WRS-1 and wild type, although the enrichments of RS were observed as expected usually. There was 1.4 times of RS enrichment in retrograded sample of mutant WRS-1, but only 1.2 times in wild type.

Table 1. Physiochemical Properties, Lipid and Protein Content in Mutant *WRS-1* and Wild Type

Materials	TS (%)	RS in Hot Flour (%)	RS In Retrograded Flour (%)	AAC (%)	LC (%)	PC (%)	Iodine-Binding Starch Properties			Thermal Properties				Crystallinity (%)	Chain-Length Distribution of Amylopectin			
							λ_{max} (nm)	BV_{680nm}	A_{620nm}	T_O (°C)	T_P (°C)	T_C (°C)	ΔH_{gel} (J/g)		DP≤12	13≤DP≤24	25≤DP≤36	DP≥37
WRS-1 (mutant)	76.8±0.3**	3.8±0.1**	5.4±0.2**	18.4±0.3*	1.7±0.1**	12.8±0.2*	591*	0.23	0.29	56.6±0.1*	61.9±0.1*	66.8±0.1*	5.0±0.1*	26.3±0.2	49.4**	38.8	7.2**	4.6**
Sumai No.6 (wild type)	69.0±0.2	0.5±0.2	0.6±0.2	15.9±0.4	2.7±0.1	16.1±0.2	586	0.20	0.26	58.8±0.0	63.9±0.1	68.5±0.1	5.2±0.1	26.6±0.2	40.4	39.0	10.4	10.2

Note: (1) TS: total starch; RS: resistant content; AAC: apparent amylose content; LC: crude lipid content; PC: protein content; BV: blue value (absorbance at λ=680nm); A620nm: absorbance at λ=620nm, where AAC was determined; T_o: onset temperature; T_c: final temperature; T_p: peak temperature; ΔH_{gel}: the enthalpy of gelatinization; DP: the degree of polymerization. (2) * and ** stand for significant differences at p0.05 and p0.01 levels, respectively, compared to that of wild type.

Correspondingly, the rate and extent of *in vitro* hydrolysis of starch were different between mutant and wild type. The starch from WRS-1, especially the retrograded sample, was highly resistant to hydrolysis and tended to be hydrolyzed in a slower rate and a lower degree than that of 'Sumai No.6' (Fig. **1**). The retrograded sample of WRS-1 needed 120 mins to reach the equilibrium of starch hydrolysis, significantly longer than other three samples. At the final time of 180 mins, only 73.8% and 79.2% of starch had been hydrolyzed in the hot and retrograded samples of WRS-1, respectively, whereas 91.8% and 94.7% of 'Sumai No.6' had been hydrolyzed.

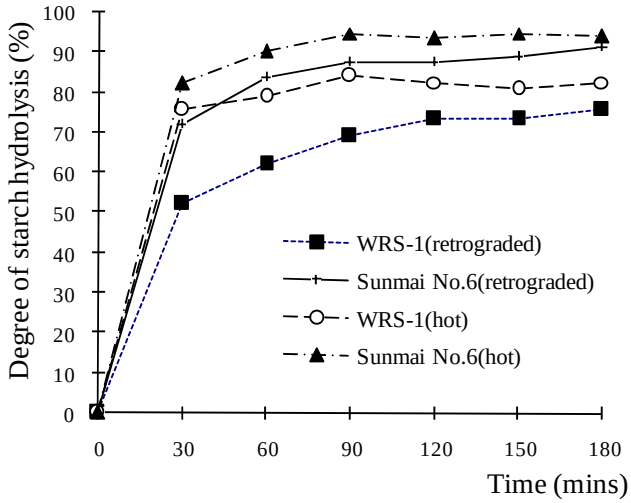

Fig. (1). *In vitro* hydrolysis of hot and retrograded flours produced from mutant WRS-1 and wild type 'Sumai No.6'.

Concurred with the previous reports in rice [5, 29], the higher level of RS and lower rate of *in vitro* hydrolysis was observed simultaneously in WRS-1. Similar to the result above and previous studies on the influences of retrogradation on RS [39-40], slow hydrolysis was observed in hot and retrograded samples.

Physicochemical Properties, Thermal Properties and X-Ray Diffraction

Higher contents of the TS and AAC were detected in mutant WRS-1, compared to that of wild type (Table **1**). This result was consistent with the previous studies that high RS was reported to correspond with relatively high levels of amylose in rice, corn, wheat, and barley [4, 14, 21, 41]. Different from the previous reports that high amylose starch was found more prone to form amylose-lipid complex, and the enhancement of RS might be caused both by the increased amylose starch and by amylose-lipid complex in mutant rice RS111 (*5*), LC and PC were obviously decreased in WRS-1.

The DSC parameters T_o, T_p, T_c and ΔH_{gel} of mutant were lower than that of wild type (Table **1**), however the decreased extent of DSC parameters in WRS-1 seemed to be somewhat different from characteristics in mutant rice high in RS.

As denoted by its characteristic reflections at 2θ, the X-ray diffraction of wheat flour showed the typical A-type crystalline pattern. The cystallinity in WRS-1 was low than that of wild type (Table **1** and Fig. (**2**)). The high degree of cystallinity needed high transition temperatures to disorder the crystal. Relatively low cystallinity in WRS-1 supported the above results on thermal properties.

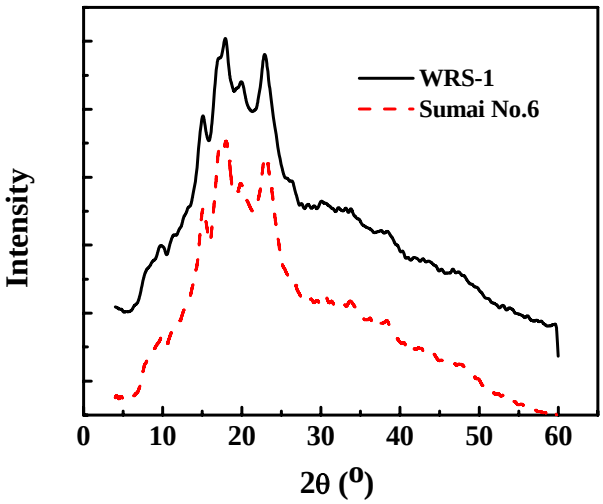

Fig. (2). X-ray diffraction pattern of flours from mutant WRS-1 and wild type 'Sumai No.6'.

Starch Granule Morphology

Scanning electron micrographs (SEM) showed that both mutant and wild type had two types of starch granules, large A in lenticular shape and small B in spherical shape as the previous report [42], whereas a few irregular-shaped granules were observed in WRS-1 (Table **2** and Fig. (**3**)). The average diameters of A- and B-shaped granules in WRS-1 were 19.5 μm and 6.6 μm, respectively, relatively larger than that of wild type that had A-shaped in an average diameters of 17.2 μm and B-shaped in an average diameters of 4.9 μm. The result above from SEM was consistent with the previous reports in which significantly higher proportion of larger starch granule was observed in some wheat variety [43]. The larger starch granules were more resistant to enzymatic digestion and the irregular granule was a typical characteristic of high RS [44].

Table 2. Shape and Size of Starch Granule in Mutant WRS-1 and Wild Type 'Sumai No.6'

Materials	No. Granules Observed	Shape Type of Starch Granule	Range of Lenticular Shape (μm)	Average Size of Lenticular Shape (μm)	Range of Spherical Shape (μm)	Average Size of Spherical Shape (μm)
WRS-1 (mutant)	481	Lenticular, spherical and irregular	12.5 to 34.7	19.5	2.8 to 9.1	6.6
Sumai No.6 (wild type)	502	Lenticular and spherical	11.7 to 32.5	17.2	1.3 to 8.5	4.9

Chain Length of Amylopectin

Based on the degree of polymerization (DP), the side chains of amylopectin can be classified into four fractions such as DP≤12, 13≤DP≤24, 25≤DP≤36 and 37≤DP, which corresponds to A, B1, B2, B3 and longer chains [36]. Compared to wild type, mutant WRS-1 had the increased intermediate and long chains with 18≤DP and decreased short chains with DP≤17, especially 8≤DP≤10 (Table **1** and Fig. (**4**)). This result was also similar to the previous reports by Shi *et al.* [45] and Salomonsson *et al.* [46], in which short chains with 6≤DP≤19 were found to inhibit the retrogradation of starch, and the higher percentage of long chains of amylopectin was observed in high-amylose barley. The chain-length profile of amylopectin in WRS-1 was the same as the previous reports on the *ae* mutants in rice [47-48]. However, the amylopectin chain ratio (ACR, expressed by the ratio of total short chains with DP≤10 to total intermediate chains with DP≤24) was significantly lower in the mutant WRS-1 with an ACR value of 0.55 compared to the wild type with an ACR value of 0.66. According to the group classification described by Nakamura *et al*. [49], WRS-1 and wild type should be different types of amylopectin. Except the ACR, the discrepancy between WRS-1 and *ae* could be also reflected from the above *To*。

The Iodine-Binding Starch Properties

In mutant WRS-1, the iodine-binding starch complex had a higher λmax of absorbance in 591 nm, blue value (BV), and value of BV620nm minus BV680nm than that of wild type (Table **1**). The absorbance at λ=620nm where AAC was determined was also higher in WRS-1 as mentioned in the result above. The current concept on AAC described in the present study is composed actually of two components: amylose and partly branched long chains (B chains) of amylopectin. The higher AAC in WRS-1 was consistent with the increased long-chain distribution of amylopectin.

CONCLUSIONS

High RS wheat was characterized by the higher RS content and slower and incomplete starch hydrolysis in both of hot and retrograded flours. The RS increase in WRS-1 is resulted significantly from starch properties and distinct starch structure such as higher AAC and TS, larger-and irregular-shaped starch granules, increased intermediate and long chains with 18≤DP and decreased short chains with DP≤17, and lower T_o, T_p, T_c, ΔH_{gel}.

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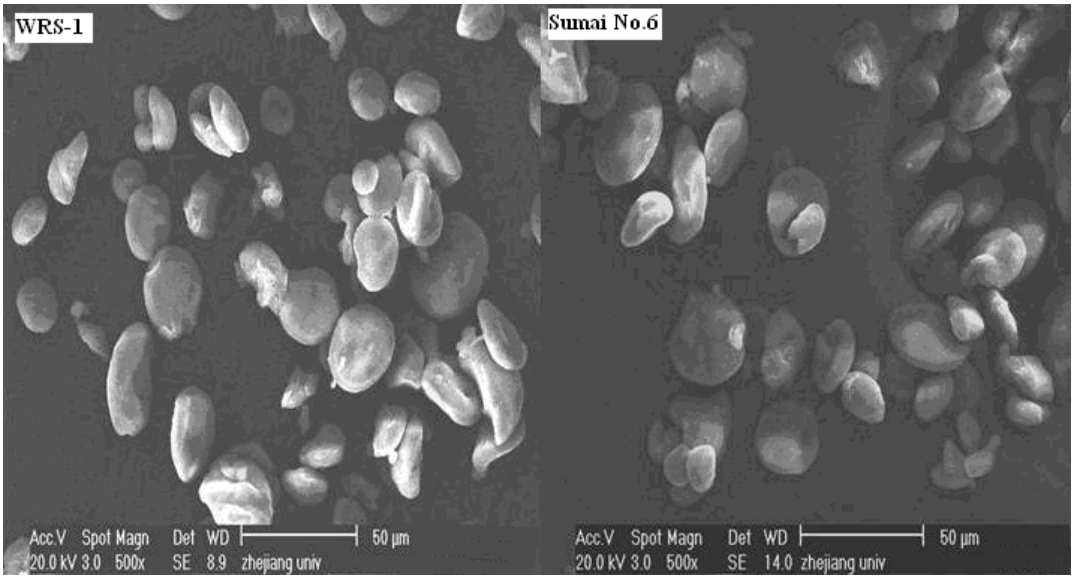

Fig. (3). Scanning electron micrographs (SEM) of starch granules from mutant WRS-1 and wild type 'Sumai No.6' (×500).

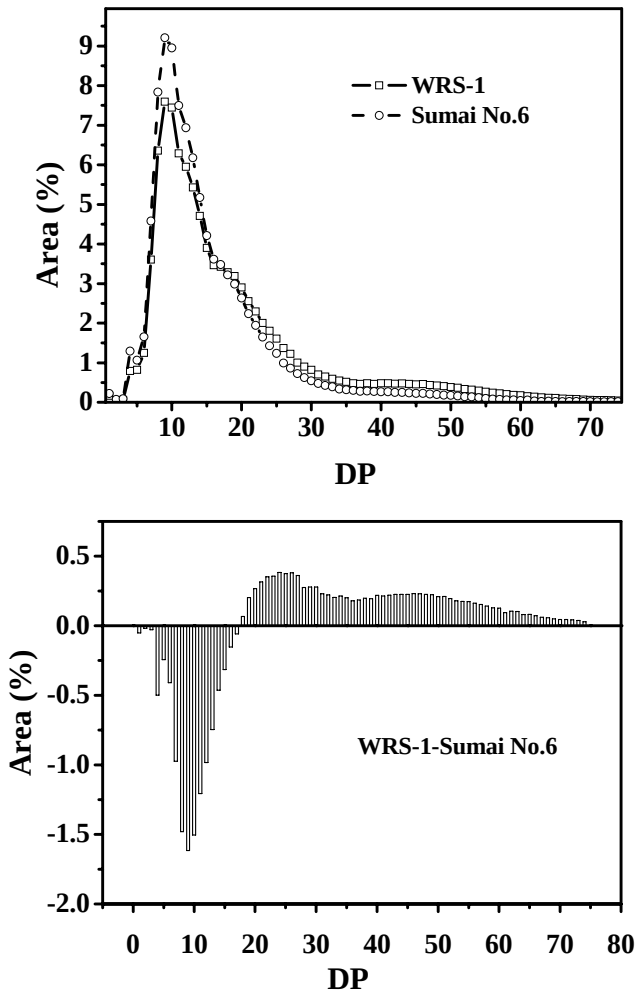

Fig. (4). Chain length of amylopectin in mutant WRS-1 and wild type 'Sumai No.6'.

ABBREVIATIONS

AAC	=	Apparent amylose content
ACR	=	Amylopectin chain ratio
BV	=	Blue value
DSC	=	Differential scanning calorimetry
DP	=	Degree of polymerization
SEM	=	Scanning electron micrographs
T_c	=	Final temperature
LC	=	Lipid content
T_o	=	Onset temperature
T_p	=	Peak temperature
PC	=	Protein content
RS	=	Resistant starch
ΔH_{gel}	=	The enthalpy of gelatinization
TS	=	Total starch

REFERENCES

[1] Jobbing S. Improving starch for food and industrial applications. Curr Opin Plant Biol 2004; 7: 210-218.

[2] James MG, Deneyer K, Myers A. Starch synthesis in cereal endosperm. Curr Opin Plant Biol 2003; 6: 215-222.

[3] Bjorck I, Nyman M, Pedersen P, Siljestrom M, Asp NG, Eggum BO. Formation of enzyme resistant starch during autoclaving of wheat starch: Studies *in vitro* and *in vivo*. J Cereal Sci 1987; 6: 159-172.

[4] Frei M, Siddhuraju P, Becker K. Studies on the *in vitro* starch digestibility and the glycemic index of six different indigenous rice cultivars from the Philippines. Food Chem 2003; 83: 395-402.

[5] Yang CZ, Shu XL, Zhang LL, *et al*. Starch properties of mutant high in resistant starch. J Agric Food Chem 2006; 54: 523-528.

[6] Englyst HN, Kingman SM, Cummings JH. Classification and measurement of 23 nutritionally important starch fractions. Eur J Clin Nutr 1992; 46(Suppl 2): S33-S50.

[7] Asp NG, Bjock I. Resistant starch. Trend Food Sci Technol 1992; 3: 111-114.

[8] Haralampu SG. Resistant starch-a review of the physical properties and biological impact of RS3. Carbohydr Polym 2000; 41: 285-292.

[9] Kim WK, Chung MK, Kang NE, Kim MH, Park OJ. Effect of resistant starch from corn on glucose control, colonic events, and blood lipid concentrations in streptozotocin-induced diabetic rats. J Nutr Biochem 2003; 14: 166-172.

[10] Nugent AP. Health properties of resistant starch. Nutr Bull 2005; 30: 27-54.

[11] Sajilata M, Singhal RS, Kukarni PR. Resistant starch-A review. Common Rev Food Sci Food Saf 2006; 51: 1-17.

[12] Wolever TMS, Mchling C. High-carbohydrate-low-glycemic-index dietary advice improves glucose disposition index in subjects with impaired glucose tolerance. Br J Nutr 2002; 87: 477-487.

[13] Åkerberg A, Liljeberg G, Bjock I. Effects of amylose/amylopectin ratio and baking condition on resistant starch formation and glycemic indices. J Cereal Sci 1998; 28: 71-80.

[14] Bird AR, Flory C, Davies DA, Usher S, Topping DL. A novel barley cultivars (Hamalaya 292) with a specific gene mutation in starch synthase IIa raises large bowel starch and short-chain fatty acids in rats. J Nutr 2004; 134: 831-835.

[15] Brouns F, Kettlitz L, Arrigon E. Resistant starch and "the butyrate revolution". Trends Food Sci Technol 2002; 13: 251-261.

[16] Topping DL, Clifton PM. Short-chain fatty acids and human colonic function: role of resistant starch and non-starch polysaccharides. Physiol Rev 2001; 81: 1031-1064.

[17] Nandini CD, Salimath PV. Carbohydrate composition of wheat, wheat bran, sorghum and bajira with good chapatti/roti (India flat bread) making. Food Chem 2001; 73: 197-203.

[18] Berry CS. Resistant starch: Formation and measurement of starch that survives exhaustive digestion with amylolytic enzymes during the determination of dietary fiber. J Cereal Sci 1986; 4: 301-314.

[19] Eerlingen RC, Van den Broeck I, Delcour JA, Levine H. Enzyme resistant starch. VI. Influence of sugars on resistant starch formation. Cereal Chem 1994; 70: 345.

[20] Tharanathan M, Tharanathan RN. Resistant starch in wheat-based products: isolation and characterization. J Cereal Sci 2001; 34: 73-84.

[21] Regina A, Bird AR, Topping DL, *et al*. High-amylose wheat generated by RNA interference improves indices of large-bowel health in rats. PNAS 2006; 103: 3546-3551.

[22] Van Der Borght A, Goesaert H, Veraverbeke WS, Delcour JA. Fractionation of wheat and wheat flour into starch and gluten: overview of the main processes and the factors involved. J Cereal Sci 2005; 41: 221.

[23] Hoebler C, Karinthi A, Chiron H, Champ M, Barry JL. Bioavailability of starch in bread rich in amylose: metabolic responses in healthy subjects and starch structure. Eur J Clin Nutr 1999; 53: 360-366.

[24] Muri JG, Yeow EG, Keogh J, *et al*. Combining wheat bran with resistant starch has more beneficial effects on fecal indexes than does wheat bran alone. Am J Clinl Nutr 2004; 79: 1020-1028.

[25] Anderson JW, Jones AE, Riddell-mason S. Ten different fibers have significantly different effects on serum and liver lipids of cholesterol-fed rats. J Nutr 1994; 124: 78-83.

[26] Behall KM, Scholfield DJ, Hallfrisch J. The effect of particle size of whole-grain flour on plasma glucose, insulin, glucagon and thyroid-stimulating hormone in humans. J Am College Nutr 1999; 18(6): 591-597.

[27] Jensen MK, Koh-Banerjee P, Franz M, Sampson L, Gronbaek M, Rimm EB. Whole grains, bran, and germ in relation to homocysterine and markers of glycemic control, lipids, and inflammation. Am J Clin Nutr 2006; 83: 275-283.

[28] Brennan CS. Dietary fibre, glycaemic response, and diabetes. Mol Nutr Food Res 2005; 49: 560-570.

[29] Shu XL, Jiao GA, Fitzgerald MA, Yang CZ, Shu QY, Wu DX. Starch structure and digestibility of rice high in resistant starch. Starch/Stärke 2006; 58: 411-417.

[30] Verwimp T, Vandepytte GE, Marrant K. Isolation and characterization of rye starch. J Cereal Sci 2004; 39: 85-90.

[31] Juliano BO, Pascual C. Quality characteristics of milled rice grown in different countries. IRRI Res Paper Ser 1980; 48: 25.

[32] American Association of Cereal Chemists (AACC). 1995. Approved methods the AACC, Ninth Edition, Method 08-10. St. Paul, MN: The Association.

[33] Garcia-Alonso A, Jimenez-Escrig A, Martin-Carron N. Assessment of some parameters involved in the gelatinization and retrogradation of starch. Food Chem 1999; 66: 181-187.

[34] Goñi I, Garcia-Alonso A, Saura-Calixto F. A starch hydrolysis procedure to estimate glycemic index. Nutr Res 1997; 17: 427-437.

[35] Umemoto T, Nakamura Y, Satoh H, Terashima K. Differences in amylopectin structure between two rice varieties in relation to the effects of temperature during grain filling. Starch/Stärke 1999; 51: 58-62.

[36] Nakamura Y, Kubo A, Shimamune T, Matsuda T, Harada K, Satoh H. Correlation between activities of starch debranching enzyme and a-polyglucan structure in endosperm of sugary-1 mutants of rice. Plant J 1997; 12: 143-153.

[37] Englyst KN, Englyst HN, Cumming JH. Rapidly available glucose in foods: an *in vitro* measurement that reflects the glycemic response. Am J Clin Nutr 1999; 69: 448-454.

[38] Jenkins DJA, Thomas DM, Wolver TMS. Glycemic index of foods: a physiological basis for carbohydrate exchange. Am J Clin Nutr 1981; 34: 362-366.

[39] Gidley ML, Cooke O, Darks AH, Holffmann RA, Rwssel AL, Greenwell P. Molecular order and structure in enzyme resistant retrograded starch. Carbohydr Polym 1995; 28: 23-31.

[40] Rashmi S, Urooj A. Effect of processing on nutritionally important starch fractions in rice varieties. Int J Food Sci Nutr 2003; 54: 27-36.

[41] Hu PS, Zhao HJ, Zhang LL, Duan ZY, Wu DX. Starch digestibility and the estimated glycemic score of different types of rice differing in amylose contents. J Cereal Sci 2004; 40: 231-237.

[42] Baum BR, Bailey LG. A survey of endosperm starch granules in the genus Hodeum: a study using image analytic and numerical taxonomic techniques. Can J Bot 1987; 65: 1563-1569.

[43] Peterson DG, Fulcher RG. Variation in Minnesuta HRS wheats: starch granule size distribution. Food Res Int 2001; 34: 357-363.

[44] Singh N, Kaur L, Sandhu KS, Kaur J, Nishiari K. Relationships between physicochemical, morphological, thermal, theological properties of rice starches. Food Hydrocolloids 2006; 20: 532-542.

[45] Shi YC, Seib PA. The structure of four waxy starches related to gelatinization and retrogradation. Carbohydr Res 1992; 227: 131-145.

[46] Salomonsson AC, Sundberg B. Amylose content and chain profile of amylopectin from normal, high amylose and waxy barleys. Starch/Starke 1994; 46: 325-328.

[47] Nishi A, Nakamura Y, Tanaka N, Satoh H. Biochemical and genetic analysis of the effects of amylose-extender mutation in rice endosperm. Plant Physiol 2001; 127: 459-472.

[48] Satoh H, Nishi A, Yamashita K, *et al.* Starch-branching enzyme I-deficient mutation specifically affects the structure and properties of starch in rice endosperm. Plant Physiol 2003; 133: 1111-1121.

[49] Nakamura Y, Sakurai A, Inaba Y, Kimura K, Iwasawa N, Nagamine T. The fine structure of amylopectin in endosperm from Asian cultivated rice can be largely classified into two classes. Starch/Stärke 2002; 54: 117-131.

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