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# The physicochemical properties and in vitro digestibility of selected cereals, tubers and legumes grown in China

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#### Abstract

Digestibility, gelatinization, retrogradation and pasting properties of starch in various cereal, tuber and legume flours were determined. Rapidly and slowly digestible starch and resistant starch were present in 11 selected flours. In general, cereal starches were more digestible than legume starches and tuber starches contained a high amount of resistant starch. Thermal and rheological properties of flours were different depending on the crop source.

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## 1. Introduction

Obesity, cardiovascular disease, diabetes and cancer are major threats to human health in North America and other industrialized nations. Although the etiology is multifactorial, diet has been identified as the single most important contributing environmental factor to the development of these diseases. Thus, dietary modification could be a costeffective way to reduce prevalence of these conditions. Increased dietary fiber intake and the possibly associated slower carbohydrate absorption are dietary recommendations accepted for potentially reducing the risk of disease development.

Cereal grains, tubers and legume seeds are staple foods in both developed and developing countries. All contain starch, but the starch digestibility is greatly influenced by plant type and depends on physicochemical characteristics of the starch and plant microstructure and composition, and is influenced by processing and

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storage conditions ([Kingman & Englyst, 1994; Ring,](#page-7-0) [Gee, Whittam, Orford, & Johnson, 1988](#page-7-0)). Most starchrelated foods are cooked before consumption and consequent starch gelatinization and retrogradation play important roles in the quality and digestibility of the many resultant food products.

There have been many reports on starch digestibility from different plant sources [\(Botham, Morris, Noel, &](#page-6-0) [Ring, 1996; Hu, Zhao, Duan, Zhang, & Wu, 2004; Madhu](#page-6-0)[sudhan & Tharanathan, 1995; van der Merwe, Erasmus, &](#page-6-0) [Taylor, 2001](#page-6-0)), but there is little information on the relationship between the starch digestibility, and the thermal and rheological properties of cereals, tubers and legumes grown in China. Glycemic index is greatly influenced by the starch digestibility (e.g. rate of starch digestion) in the food system. Resistant starch and slowly digestible starch result in low glycemic index in starch-based food products. In recent years, the glycemic index has been transformed from a potentially useful tool in planning diets for diabetic patients to a key player in the prevention of diabetes, hyperlipidemia, cardiovascular disease, and even certain types of cancer in the general population (Björck  $\&$  Asp,

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[1994](#page-6-0)). However, no information is available on quickly digestible starch, slowly digestible starch and resistant starch in vitro in cereals, tubers and legumes grown in China. In addition, the retrogradation behaviour of selected plant flours has not been fully investigated and understood.

The aims of this study were to determine starch digestibility in flours that were used in an animal study from a national research program in China, and to characterize the gelatinization, retrogradation and pasting properties of these flours.

#### 2. Material and experimental methods

# 2.1. Flours

The flours of corn, millet, oat, wheat, rice, buckwheat, potato, sweet potato, taro, mung bean and broad bean were provided by the Institute of Subtropical Agriculture, The Chinese Academy of Science, Changsha, Hunan, China. These flours were directly fed to pigs as major energy sources to study the physiological response from pig. The sources of plant materials were: corn (Northern Crop Breeding Company, Jinlin, China), millet (Shan Xi, Crop Breeding Company, Shan Xi, China), oat (Northern Crop Breeding Company, Jinlin, China), wheat (Hunan Crop breeding company, Hunan, China), rice (Hunan Crop Breeding Company, Hunan, China), buckwheat (Northern Crop Breeding Company, Jinlin, China), potato (Hubei Crop Breeding Company, Hubei, China), sweet potato and taro (Hunan Crop Breeding Company, Hunan, China), mung bean and broad bean (Shan Xi Crop Breeding Company, Shan Xi, China). Flour preparations were carried out based on published procedures in Institute of Subtropical Agriculture, The Chinese Academy of Science, Changsha, Hunan, China. The flours were stored in plastic bags at room temperature  $(22 \degree C)$  and used in experiments as received.

The chemicals and pancreatic  $\alpha$ -amylase (pancreatin P-1625) were purchased from Sigma Chemical Co., St. Louis, MO, USA. a-Amylase (Megazyme E-BLAAM) and amyloglucosidase (E-AMGDF) were purchased from Megazyme (Megazyme International Ireland Ltd., Bray, Ireland).

## 2.1.1. Moisture content

Moisture content of flours was determined in duplicate. Samples  $(\sim 0.2 \text{ g})$  were weighed in aluminum pans before and after drying at  $85^{\circ}$ C and  $27$  in. Hg vacuum for  $24$  h. Samples were removed from the oven and immediately placed in a desiccator prior to weighing after cooling and within 20 min.

# 2.1.2. Total starch content

Starch content of flour was determined based on [AACC](#page-6-0) [\(2000\)](#page-6-0) method 76.13 B. To 100 mg flour,  $3 \text{ mL } \alpha$ -amylase solution (300 U), in 50 mM MOPS buffer (pH 7.0) were added. The sample was heated in a boiling water bath for 6 min with constant stirring, and then was cooled to below 50 °C. One hundred microlitres (20 U) amyloglucosidase and 4.0 mL 200 mM sodium acetate buffer (pH 4.5) were added to the sample. The sample was mixed well and incubated at 50  $\degree$ C for 30 min with constant stirring. Wheat flour from Megazyme was employed as a standard in every batch experiment to verify enzyme activity. Blank samples (without enzymes) were also measured with the same procedure. The reported values are averages of duplicate measurements.

#### 2.1.3. Digestibility

Flour (100 mg) was incubated with pancreatic  $\alpha$ -amylase (10 mg) and amyloglucosidase (AMG) (12 U) in 4 mL of a 0.1 M sodium maleate buffer, pH 6.0, in a shaking water bath at  $37^{\circ}$ C for  $30$  min, 2 and 16 h. The resistant and non-resistant starch contents were measured using a modified Megazyme procedure. Kidney beans with a known RS content were used as a standard in each experiment.

Hydrolysis of non-resistant starch. Ethanol (95%) was added after incubation, the sample was then centrifuged and the pellet washed with 50% ethanol. The supernatant was collected and diluted to a fixed volume for glucose content measurement. The non-resistant starch content was determined from glucose content in the supernatant using a YSI 2700 Select Biochemistry Analyzer (Yellow Springs Incorporated, Yellow Springs, OH, USA).

Measurement of resistant starch. The residue (pellet) was dissolved in KOH for 20 min in an ice-water bath with stirring. Concentrated AMG (330 U) was added and the pellet was incubated for 30 min at 50  $\degree$ C with stirring. Glucose content of the hydrolysate was measured by YSI 2700 and resistant starch content was determined.

Digestibility is expressed as the ratio of total non-resistant starch to the sum of resistant starch and non-resistant starch.

#### 2.2. Differential scanning calorimetry (DSC)

Thermal analyses were carried out using a differential scanning calorimeter (2920 Modulated DSC; TA Instruments, New Castle, DE, USA) equipped with a refrigerated cooling system (RCS). Samples of flour were weighed into high-volume pans. Distilled water was added using a micropipette to make 70% moisture content. Sample weights were about 12 mg. Pans were sealed and equilibrated overnight at room temperature before heating in the DSC. The measurements were carried out using a heating rate of  $10 \degree C/$ min between 5 and 180 °C. The instrument was calibrated using indium and an empty pan as a reference. The enthalpy  $(\Delta H)$  of phase transitions was measured from the endotherm of DSC thermograms using software (Universal Analysis, Version 2.6D, TA Instruments) based on the mass of dry solid. Onset  $(T_0)$  and peak temperature  $(T_n)$  of endotherms were also measured from the thermograms.

*Retrograded flours:* After heating to  $180^{\circ}$ C, samples were cooled to 5 °C. Once the temperature reached 5 °C, the sample was immediately removed from the DSC and

<span id="page-2-0"></span>stored at  $5^{\circ}$ C. After about 2 weeks, stored samples were reheated from 5 to 180 °C at 10 °C/min. The enthalpy  $(\Delta H)$ , onset temperature  $(T_o)$  and peak temperature  $(T_p)$  of the endotherm were measured from the thermograms based on dry solid mass. The reported values are the means of duplicate measurements.

## 2.3. Rapid viscosity analysis (RVA)

The pasting properties of the flours  $(11.9\% \text{ dsb}, 29 \text{ g to-}$ tal weight) were measured using a Rapid Visco<sup> $m$ </sup> Analyser RVA-4 (Newport Scientific Pty. Ltd., Warriewood, NSW, Australia). The STD 2 profile (AACC method 76–21) [\(AACC, 2000](#page-6-0)), in which the sample is equilibrated at 50 °C for 1 min, heated at 6 °C/min to 95 °C, held at 95 °C for 5 min, cooled at 6 °C/min to 50 °C, and held at 50 °C for 2 min was used. The speed was 960 rpm for the first 10 s, then 160 rpm for the remainder of the experiment. Peak viscosity, final viscosity and pasting temperature of these flours were compared from pasting curve. The reported values are means of duplicate measurements.

## 3. Results and discussion

## 3.1. Total starch content of flours

Starch was a major component of the flours (Table 1) varying from 34% to 85% on a dry weight basis (dwb). Pulse flours contained much less starch than the cereal and tuber flours. Most cereal flours had higher starch content than tuber flours. The sum of RS and non-RS was similar to total starch by the AACC method as shown in Table 1; however, differences exist. This may be due to interference between starch and non-starch polysaccharides and other components such as lipids and proteins during different enzyme hydrolysis in the two methods.

## 3.2. Starch digestibility

[Table 2](#page-3-0) shows the resistant and non-resistant starch content of selected flours at different incubation times. Rapidly available glucose (RAG) was measured after incubation

Table 1 Total starch and moisture content  $(\% , w/w)$  of selected flours

Flour	By AACC method	Sum of RS and non-RS	Moisture content
Corn	$60.0 \pm 4.6$	65.2	$10.3 \pm 0.4$
Millet	$73.5 \pm 1.0$	75.9	$10.6 \pm 0.3$
Oat	$65.0 \pm 0.9$	57.3	$10.2 \pm 0.5$
Rice	$85.2 \pm 3.6$	81.4	$7.6 \pm 0.7$
Wheat	$69.6 \pm 5.7$	71.6	$10.4 \pm 0.0$
<b>Buckwheat</b>	$67.2 \pm 2.7$	64.9	$9.3 \pm 0.0$
Potato	$63.8 \pm 0.1$	60.6	$11.8 \pm 0.2$
Sweet potato	$64.4 \pm 1.6$	60.3	$7.4 \pm 0.1$
Taro	$60.7 \pm 2.4$	56.6	$9.9 \pm 0.0$
Mung bean	$34.1 \pm 1.0$	37.3	$10.9 \pm 0.4$
Broad bean	$34.6 \pm 4.1$	34.8	$7.5 \pm 0.1$

with a mixture of pancreatic  $\alpha$ -amylase and amyloglucosidase at  $37^{\circ}$ C. A value for RAG was obtained as glucose released from the food commonly after 20 min  $(G_{20})$ , but we used 30 min to measure the RS and non-RS. A second measurement of RS and non-RS was obtained after a further 90 min incubation. Based on the glucose released after 120 min, one can determine slowly digestible starch. A third measurement was obtained after 16 h incubation. This measurement allows one to evaluate resistant starch content in the food and food products.

The resistant starch content after 30 min incubation followed the order:  $Rice > Wheat > Potato > Taro >$ Buckwheat > Millet > Corn > Sweet potato > Broad bean > Mung bean > Oat. Oat flour contained very little resistant starch (3.1%). The resistant starch content after 120 min incubation followed the order: Wheat > Potato > Taro > Rice > Sweet potato > Corn > Broad bean > Millet > Buckwheat > Mung bean > Oat. After 16 h incubation, the resistant starch contents were 49.3%, 42.1%, 6.4%, 4.0%, 3.8% and 3.4% for potato, taro, corn, broad bean, mung bean and sweet potato, respectively. The resistant starch contents in millet, rice, oat, and wheat buckwheat flour were less than 1%.

[Fig. 1](#page-3-0) presents the digestibility of these flours as a function of incubation time.

From the results of [Fig. 1](#page-3-0) and [Table 2](#page-3-0), it is clear that the order of quickly digestible starch in the flour is  $\text{Oat}$ Mung bean > Buckwheat; Millet; Sweet potato > Corn; Rice > Broad bean; Wheat; Taro and Potato. Rapidly digestible starch is rapidly and completely digested in the small intestine. It is associated with more rapid elevation of postprandial plasma glucose and insulin. Slowly digestible starch is completely but more slowly digested in the small intestine and leads to attenuated postprandial plasma glucose and insulin levels; it is generally the more desirable form of dietary starch ([Jenkins et al., 1981](#page-7-0)).

The order of slowly digestible starch in the flours is Potato > Taro > Wheat > Broad bean > Corn; Rice; Sweet potato > Mung bean > Millet > Buckwheat > Oat [\(Table](#page-3-0) [2\)](#page-3-0). The most resistant starches were in potato and taro flour with RS content of 49.3 and 42.1, respectively. As shown in [Fig. 1](#page-3-0) and [Table 2,](#page-3-0) RS was almost non-detectable in the flours of millet, oat, rice, wheat and buckwheat. Different amounts of digestible starch in selected flours could arise from heat treatments during the processing of flour. Microscopy showed loss of birefringence of starch in oat flour, but not in wheat flour (data not shown) indicating some granule disruption during processing. The unusual level of RS (6.4%) in the corn flour (relative to Ontario values of 2%) presumably arises from the source, either varietal, environmental or processing.

# 3.3. Thermal behaviour of flours from various sources

#### 3.3.1. Initial heating of flour at moisture content of 70%

When flours were heated in the presence of excess water (70%), an endothermic transition was observed, as shown

<span id="page-3-0"></span>Table 2 Resistant and non-resistant starch content of flour at different incubation times

	30 min incubation		120 min incubation		16 h incubation	
	$RSa(\%)$	Non-RS $(\% )$	$RS(\%)$	Non-RS $(\% )$	$RS(\%)$	Non-RS $(\%$
Corn	$47.2 \pm 1.6$	$8.5 + 0.8$	$29.8 + 2.5$	$23.1 \pm 0.3$	$6.4 \pm 0.1$	$58.8 \pm 0.9$
Millet	$49.8 + 0.1$	$12.3 \pm 0.2$	$17.2 \pm 0.5$	$32.3 \pm 0.4$	$0.2 \pm 0.2$	$75.7 \pm 1.1$
Oat	$3.1 \pm 0.1$	$17.3 \pm 0.6$	$0.6 \pm 0.1$	$30.5 \pm 0.4$	$0.4 \pm 0.1$	$56.9 \pm 0.7$
Rice	$59.5 + 0.4$	$11.4 + 0.8$	$40.3 + 1.0$	$28.9 + 1.1$	$0.6 \pm 0.3$	$80.8 \pm 0.6$
Wheat	$58.8 \pm 1.7$	$7.6 \pm 0.0$	$52.1 \pm 0.0$	$16.5 \pm 0.3$	$0.6 + 0.1$	$70.9 \pm 0.1$
Buckwheat	$50.3 \pm 2.0$	$12.6 \pm 1.0$	$15.1 \pm 1.9$	$37.9 \pm 2.7$	$0.8 \pm 0.1$	$64.2 \pm 0.4$
Potato	$54.3 \pm 2.7$	$3.9 \pm 0.8$	$50.7 \pm 0.9$	$5.9 \pm 0.4$	$49.3 \pm 2.4$	$11.3 \pm 0.2$
Sweet potato	$42.0 \pm 1.5$	$9.2 + 0.4$	$31.3 + 4.6$	$20.3 \pm 1.0$	$3.4 + 0.0$	$57.0 + 0.4$
Taro	$51.5 \pm 6.5$	$5.3 \pm 0.8$	$50.4 \pm 1.2$	$9.1 \pm 0.6$	$42.1 \pm 1.7$	$14.5 \pm 1.4$
Mung bean	$21.9 \pm 2.7$	$8.0 \pm 0.2$	$14.4 \pm 1.0$	$17.6 \pm 1.3$	$3.8 \pm 0.5$	$33.5 \pm 0.8$
Broad bean	$30.1 \pm 0.6$	$3.6 \pm 0.9$	$22.6 \pm 0.7$	$10.8 \pm 0.6$	$4.0 \pm 0.7$	$30.8 \pm 0.8$

<sup>a</sup> RS; resistant starch content.



Fig. 1. Starch digestibility in various flours.

in [Fig. 2](#page-4-0). There were three different types of thermal transitions for flour from cereals, tubers and legumes and the thermal properties such as transition temperature and enthalpy were different for each flour, as presented in [Table 3.](#page-4-0)

In [Fig. 2](#page-4-0), the endothermic peak of tuber flour mainly reflected starch gelatinization ([Liu, Weber, Fan, & Yada,](#page-7-0) [2002](#page-7-0)). In tuber flours such as potato, sweet potato and taro, a single symmetrical endothermic transition was observed. However, different endothermic transitions were observed for flours from different sources. Cereal flours such as corn, rice, wheat, and buckwheat showed a major first peak at a lower temperature and a second shoulder or peak at higher temperature. The transition at lower temperature was attributed to starch gelatinization. Disruption of starch (amylose) lipid complex structures in the flour took place at a higher temperature as indicated by the appearance of a shoulder or peak at around  $100^{\circ}$ C in the DSC thermogram. The thermal behaviour of legume flours is different from tuber and cereal flours. There are two relatively small separate endothermic transitions.

[Table 3](#page-4-0) summarizes the thermal properties of the different flours.

Tuber flours had higher transition temperatures and enthalpy than cereal and legume flours. In cereal flours, the peak temperatures of the second peak were all similar indicating the melting of the starch–lipid complex. However, different enthalpies of that transition were observed. This may be due to the difference in the content and type of lipids in different cereal flours. Two legume flours showed similar thermal behaviour, but details of the thermal properties differed. Broad bean had a higher second peak temperature and enthalpy value for both endothermic transitions compared to mung bean flour. In contrast, mung bean had a slightly higher peak temperature in the first endothermic transition.

#### 3.3.2. Reheating of flour samples after 2 weeks of storage

After 2 weeks of storage at  $5^{\circ}$ C, flour samples were reheated in the DSC to investigate starch retrogradation and the factors influencing starch retrogradation. The thermal properties of retrograded flours are summarized in [Table](#page-4-0) [4.](#page-4-0) The transition temperatures were lower than starch gelatinization temperatures in the original flour. The thermograms of retrograded flour gel consisted of three distinct types as shown in [Fig. 3.](#page-5-0) The thermal behaviour of storage flour gel almost follows the pattern of flour during initial heating but to different degrees. The major endothermic peak is attributed to thermal transition of retrograded starch [\(Liu, Weber, Currie, & Yada, 2003\)](#page-7-0). Tuber flour gel had a higher peak temperature than cereal flour gel. The peak temperatures of starch retrogradation from legume flour gels were between the tuber flour gel and cereal flour gel. Rice and buckwheat showed a strong starch retrogradation peak (the first peak) in the thermograms. However, no starch retrogradation peak was observed for corn and oat flour gel ([Table 4\)](#page-4-0). Lack of a starch retrogradation endothermic peak in these flours might be caused by: (1) the interaction of other components; (2) the processing of the flour; and (3) damage of the molecular structure of the starch ([Liu & Thompson, 1998](#page-7-0)).

The endothermic peak at higher temperature ( $\sim$ 100 °C) ([Fig. 3](#page-5-0)) may be due to the presence of starch–lipid complex

<span id="page-4-0"></span>

Fig. 2. Thermograms of three types of flours in the presence of water (70%) upon heating.





 $\sqrt[3]{n}$  N/D, not detectable.





<sup>a</sup> Single measurement due to the failure of another sample.

<span id="page-5-0"></span>

Fig. 3. Typical thermograms of reheated flour samples (70% water content) after 2 week storage at 5 °C.

in the cereal and legume flour gels. However, the thermal properties of this transition were different among cereal flour gels and legume flour gels. For example, the peak temperature of the thermal transition varied from 91 to 100  $^{\circ}$ C. The enthalpy of this transition also varied among the cereal flour gels. The difference may be due to differences in starch content, lipid content, starch structure including the amount of amylose and amylopectin, molecular weight of amylose and amylopectin and its distribution, and the interaction between starch and other components.

#### 3.4. Pasting properties of flours from various sources

The pasting properties and the RVA profiles of selected flours are presented in Table 5 and [Fig. 4](#page-6-0), respectively. The pasting properties varied with flour source and sample concentration (data not shown). Cereal flours such as oat, wheat, rice and the pseudo-cereal buckwheat had a higher peak viscosity and final viscosity. Cereal flours exhibited a wider range of pasting temperature  $(54–84 \degree C)$ . The selected corn flour had the highest pasting temperature  $(84 \degree C)$ . Legume flours such as mung bean and broad bean had a lower peak viscosity and final viscosity. The peak viscosity of broad bean could not even be calculated by the software. Lower viscosity was due to the lower starch content in the flour ([Table 1](#page-2-0)). The pasting temperature of mung bean was higher than that of broad bean. Tuber flours such as potato and sweet potato had a moderate peak viscosity and final viscosity. The pasting temperatures of tuber flours were similar, ranging from 73 to 79 °C. Taro flour exhibited much lower peak and final viscosity than potato and sweet potato flour. Among the selected flours, Table 5





<sup>a</sup> Not determined.

the order of peak and final viscosity is: cereal (except corn flour)  $>$  tuber  $>$  legume.

The peak viscosity of pure potato starch was much higher than that of cereal starches because of the larger granule size and phosphorus content (in the form of phosphate monoesters) ([Jane, 2004, Chap. 7](#page-7-0)). However, the peak viscosity of potato flour showed a moderate value compared to other cereal flours. Lower starch content in the potato flour [\(Table](#page-2-0) [1\)](#page-2-0) was a major factor. Starch content in the flour, other components in the starch–water system and processing of flours are very critical to pasting properties. The interaction of other components and the degree of starch damage during processing could affect the peak viscosity of flours.

The final viscosity of starch gels is affected by starch retrogradation. Previous data indicate the retrogradation properties of flours differed by plant sources. Most cereal

<span id="page-6-0"></span>

Fig. 4. RVA profiles of selected flours: ((a) rice; (b) millet; (c) oat; (d) buckwheat; (e) wheat; (f) potato; (g) sweet potato; (h) corn; (i) taro; (j) mung bean; (k) broad bean; (l) temperature profile).

flours exhibited a second endothermic peak at a higher temperature in the DSC due to the presence of lipid in the cereal flour and lower retrogradation temperatures than tuber flours. Some cereal flours such as oat showed no starch retrogradation endothermic peaks at lower temperature, but oat flour had a higher final viscosity, indicating starch retrogradation of oat starch may not be the only factor for higher final viscosity of oat flours. In this case, the molecular structure of oat starch might play a key role in starch retrogradation. In addition,  $\beta$ -glucan could contribute to oat flour viscosity ([Wood, 2004\)](#page-7-0). Although oat flour contained about 65% starch content, the higher amount of starch in other cereal flours remained a factor for their higher final viscosity. It seems that the higher the viscosity of flour, the lower resistant starch content in the flour. However, the relationship between pasting properties and digestibility of flours is not clear for all the selected flours in this study. Nevertheless, viscosity might be an important parameter for the indication of starch digestibility in processed foods.

# 4. Conclusions

Starch digestibility in flour varied with plant source. In general, cereal flours had more rapidly digestible starch than legume and tuber flours. Tuber flours had the highest amount of resistant starch. Cereal, tuber and legume flours showed distinctive thermal behaviour. Starch gelatinization and retrogradation were observed in most flours upon heating flour–water system and stored flour gel. Pasting properties were also different among the selected flours. The order of viscosity was cereal  $>$  tuber  $>$  legume flours. Pasting properties are greatly influenced by plant source,

starch content, interaction among the components, and testing conditions. Among the selected flours, oat showed the highest amount of rapidly digestible starch. The highest peak viscosity and final viscosity were obtained from oat flour. No starch retrogradation was observed in oat flour under the experimental conditions. Different processing of flour could be another factor influencing the physicochemical properties of flour.

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