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In vitro testing indicates an accelerated rate of digestion of starch into glucose of cooked rice with the development of low amylose rice in China

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A R T I C L E I N F O

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A B S T R A C T

The consumption of low amylose rice has substantially increased in China in recent years. This *in vitro* study showed that the starch digestion process was distinctly different between a group of commercial rice samples (CR, $n = 34$) with low amylose content (14–20%) and a group of control rice samples (CK, $n = 16$) with high amylose content (24–30%). In particular, the CR group had an active digestion duration that was ~ 90% shorter and a rate of glucose production within the active digestion duration that was nearly 50% higher compared to the CK group. The findings of this study indicate that the development of low amylose rice in China can result in an acceleration in the rate of digestion of starch into glucose of cooked rice, highlighting the need for *in vivo* assessment of the potential risk of diabetes associated with the consumption of low amylose rice.

1. Introduction

China is the country with the highest number of diabetics (mainly type 2 diabetes) in the world; 116 million people had diabetes in 2019, which accounts for 25% of the global diabetes population (Saeedi et al., 2019). Moreover, it is estimated that China has a pre-diabetes population that may number as many as ~ 500 million people (Xu et al., 2013). In the Healthy China 2030 Plan released by the State Council of China, diabetes, along with cancer, hypertension, and cardiovascular diseases is listed as one of the four high priority non-communicable diseases that need to be addressed with the goals of controlling the prevalence and reducing the probability of early death (Luo et al., 2020).

There are several factors that contribute to the prevalence of type 2 diabetes in China, including changes in diet and lifestyle associated with rapid urbanization, familial factors such as inherited genetic variants, early-life factors such as maternal undernutrition, maternal obesity, and gestational diabetes, and gene-environment interactions (Ma et al., 2014). Epidemiologic studies and randomized clinical trials have shown that the risk of type 2 diabetes can be substantially reduced by diet and lifestyle modifications (Li et al., 2008; Lindström et al., 2006; Pan et al., 1997), and hence it has been suggested that primary prevention through promotion of a healthy diet and lifestyle should be a public policy priority to curb the escalating diabetes epidemic (Hu, 2011).

Rice is a high-carbohydrate staple food that feeds more than 65% of the population in China (Hsiaoping, 2005). The supply of rice has been sufficient to meet domestic demand in China due to of an increase of greater than 50% in grain yield per unit area since 1980 (Deng et al., 2019). The sufficient supply of rice has led to dietary changes such as replacing coarse cereals (cereal grains other than rice and wheat) and whole grains (*e.g.*, brown rice) with polished rice (white rice) (Hu, 2011; Ma et al., 2014). There is ample evidence that higher consumption of white rice is associated with an increased risk of type 2 diabetes in Asian populations, including the Chinese (Hu et al., 2012; Villegas et al., 2007), while intake of coarse cereals and whole grains including brown rice is inversely associated with the risk of developing type 2 diabetes (de Munter et al., 2007; Kaur et al., 2014).

More recently, the demand and consumption of high eating-quality rice, mainly low amylose rice, has substantially increased in China as living standards improve (Liu et al., 2020; Yin et al., 2020), although high eating-quality rice is sold at a relatively high price. To cater to the needs of the consumer and the rise in farmers' incomes, the improvement in eating quality has received unprecedented interest and support in rice research and production in China, and more and more new low amylose rice varieties have been developed and grown in China (Zeng et al., 2019).

Here, we worry that the rapid development of low amylose rice may go counter to controlling the diabetes epidemic in China, because rice with a lower amylose content is generally less resistant to digestion and

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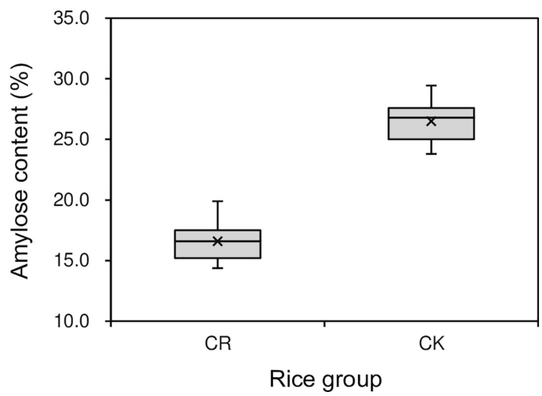

Fig. 1. Amylose content in the two experimental rice groups; samples of commercial rice purchased from the market in Changsha, Hunan Province, China (CR, $n = 34$) and a group of control rice samples provided by the Crop and Environment Research Center for Human Health at Hunan Agricultural University, China (CK, $n = 16$). The box-whisker diagram shows the minimum (end of the lower whisker), 25th percentile (lower edge of the box), mean (cross within the box), median (horizontal line within the box), 75th percentile (upper edge of the box), and maximum (end of the upper whisker) values of the data.

Table 1
Names, origins, and release dates of rice samples included in the control (CK) group.

Sample No.	Variety name	Type	Origin	Release year
1	Guangluai 4	Inbred	Guangchangai 3784/ Lucaihao	1967
2	Guichao 2	Inbred	Guiyangai 49/ Chaoyangzao 18	1973
3	Guiliangyou 2	Hybrid	Guike-2S/Guihui582	2008
4	Liangyoupeijiu	Hybrid	Peiai 64S/93–11	1999
5	Luliangyou 996	Hybrid	Lu 18S/996	2005
6	Tianyouhuazhan	Hybrid	Tianfeng A/Huazhan	2008
7	Wufengyou T025	Hybrid	Wufeng A/Changhui T025	2008
8	Wuyou 308	Hybrid	Wufeng A/Guanghui 308	2004
9	Xiangzaoxian 24	Inbred	Xiangzaoxian 11/ Xiangzaoxian 7	1995
10	Yuxiangyouzhan	Inbred	TY36/IR64//IR100	2005
11	Zhenguiai 1	Inbred	Zhenyeai/Guiqing 3	1990
12	Zhongjiazao 17	Inbred	Zhongxuan 181/Jiayu 253	2008
13	Zhongzao 39	Inbred	Jiayu 253/Zhongzu 3	2009
14	Zhuliangyou 729	Hybrid	Zhu 1S/E7299	2011
15	E1703	Inbred	Xiangzao 33/GER-2	Not-released
16	Yuezaoxian 17	Inbred	Xiangzao 33/GER-1	Not-released

has a higher glycemic index (Fitzgerald et al., 2011; Frei et al., 2003; Hu et al., 2004). However, there is limited direct information on the digestion properties of low amylose content rice consumed in China. To bridge this information gap, this study determined the starch digestion characteristics of a group of low amylose rice samples purchased from the market in China by comparing them with a group of rice samples with high amylose content. This work will not only provide valuable information to promote a healthy population in China but is also of great significance for the other major rice-consuming countries.

2. Materials and methods

2.1. Rice samples

Two groups of rice samples, a commercial rice (CR) group and a control rice (CK) group, were used in this study. The CR group included 34 rice samples purchased from the market in Changsha, Hunan Province, China. The rice samples in the CR group were produced in the Chinese provinces of Guangdong ($n = 2$), Hubei ($n = 10$), Hunan ($n = 19$), and Jiangxi ($n = 3$).

Amylose content in the CR group rice samples ranged from 14% to 20%, with a mean of 17% (Fig. 1). The CK group included 16 rice samples provided by the Crop and Environment Research Center for Human Health at Hunan Agricultural University, China (Table 1). The minimum and maximum amylose contents in the CK group samples were 24% and 30%, respectively, with a mean of 27% (Fig. 1).

The amylose content of the rice samples was measured using a spectrophotometric method and adjusted to a moisture content of 14%. Polished rice grains were ground into flour and passed through a 100-mesh sieve. Approximately 100 mg of the sieved rice flour was dispersed in 1 mL of 95% ethanol, gelatinized by adding 9 mL of 1 mol L^{-1} NaOH in a boiling water bath for 10 min, and then diluted to 100 mL with distilled water. A color reaction was developed by taking 2.5 mL of the diluted solution, adding 0.5 mL of 1 mol L^{-1} acetic acid and 1 mL of iodine solution containing 2% KI and 0.2% I_2, and then diluting to 50 mL with distilled water. The absorbance of the colored solution was measured at 620 nm with an L8 Double Beam UV–VIS spectrophotometer (INESA Analytical Instrument Co., Ltd., Shanghai, China). A standard curve was generated using a series of standard solutions with different amylose concentrations that was provided by the Rice Product Quality Inspection and Supervision Testing Center of the Ministry of Agriculture of China to calculate the amylose contents in the experimental rice samples.

2.2. Starch digestion testing

The starch digestion properties of the cooked polished rice were determined by an *in vitro* method. About 10 g of polished rice was soaked in 16 mL of distilled water in an aluminum cup for 30 min, and the aluminum cup was then covered with its lid and placed in an electric rice cooker (GDF-2003, Zhuhai Gree Group Co., Ltd., Zhuhai, China)

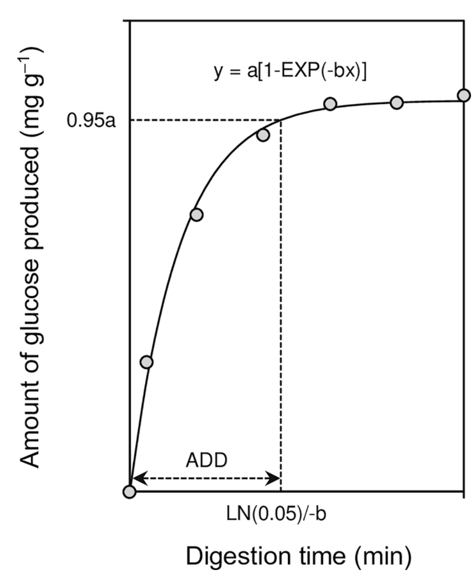

Fig. 2. A schematic diagram for fitting the kinetics of starch digestion in cooked rice to the exponential association model, y = a[1-EXP(-bx)], where y is the estimated amount of glucose produced per unit fresh weight of cooked rice from digestion, x is the digestion time, a is the estimate of the final amount of glucose produced, and b is a parameter related to the active digestion duration (ADD), which was estimated with y at 95% of a (0.95a) and solving for × using the equation x = LN(0.05)/−b. Each circle represents the observed amount of glucose produced at a given digestion time.

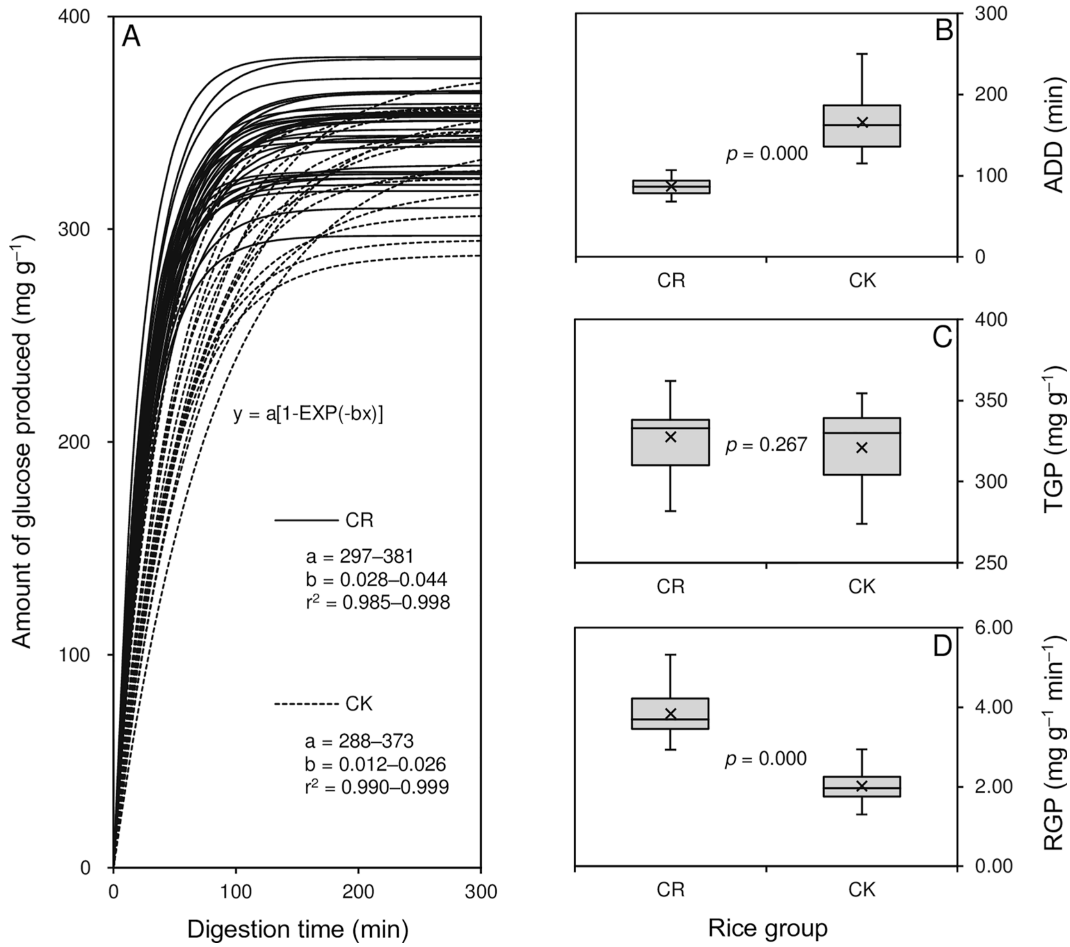

Fig. 3. The starch digestion process fitted to the exponential association model (A), estimated digestion parameters, including active digestion duration (ADD) (B), total glucose production (TGP) (C), and the rate of glucose production (RGP) (D) within the ADD, in the CR and CK groups of rice samples (see legend to Fig. 1 for sample details). The box-whisker diagram shows the minimum (end of the lower whisker), 25th percentile (lower edge of the box), mean (cross within the box), median (horizontal line within the box), 75th percentile (upper edge of the box), and maximum (end of the upper whisker) values of the data. The *p*-value is for the comparison of the means between the CR and CK groups based on the two-sample *t* test.

containing approximately 700 mL of boiling water and allowed to steam for 40 min. The lid of the electric rice cooker remained locked for 20 min after steaming finished. The cooked rice was immediately chopped 20 times by a hand-held food chopper (Zyliss, Zurich, Switzerland) to simulate human chewing (Woolnough et al., 2008). Three subsamples (replicates) of ∼ 100 mg of the chopped rice was subjected to *in vitro* digestion to determine the amount of glucose produced per unit fresh weight of cooked rice at different digestion times (15, 60, 120, 180, 240, and 300 min) using a Glycemic Index Analyser (NutriScan GI20; Next Instruments, Condell Park, New South Wales, Australia) (Chandel et al., 2016). In brief, samples were transferred into the sample cups of the NutriScan GI20 that contains a stirrer bar and kept in a heating block to maintain the temperature to 37 °C. Three enzyme solutions, *i.e.*, 2 mL of α-amylase solution (250 U mL^{-1}), 5 mL of pepsin solution (1 mg mL^{-1}), and 5 mL of pancreatin and amyloglucosidase solution (dissolving 130 mg of pancreatin and 58.8 mg of amyloglucosidase by 120 mL of 0.2 M sodium acetate buffer with pH 6.0), were added to each sample cup in sequence at a certain time interval. Samples were digested as occurs inside the human system of the NutriScan GI20 and the amount of glucose produced from these digested samples was measured at successive intervals during the digestion process. The expression of glucose production amount in a basis of food (cooked rice) fresh weight was selected according to the opinion of Monro (2003), who stated that the food-based index is more practical than the carbohydrate-based index for dietary management.

The starch digestion of the cooked rice (the change in the amount of glucose produced over time) was fitted to the exponential association model (Huang et al., 2021; Fig. 2). The fitting was done using CurveExpert 1.4 software (Hyams Development, Chattanooga, TN, USA). Digestion parameters of the cooked rice, including active digestion duration (ADD), total glucose production (TGP), and the rate of glucose production (RGP) within the ADD, were estimated using the following formulas: ADD = LN(0.05)/-b; TGP = 0.95a; and RGP = TGP/ADD, where a and b were obtained from the fitting.

2.3. Statistical analysis

The data for the digestion parameters including ADD, TGP, and RGP of each rice group were subjected to the Shapiro-Wilk test of normality (Statistix 8.0, Analytical Software, Tallahasse, FL, USA), which showed that all the data had a normal distribution ($p = 0.094$–0.588). Therefore, no data transformation was required, and the mean values of each parameter were compared between the CR and CK groups using the two-sample *t* test after a homogeneity of variance test (DPS 18.10, Analytical Software, Hangzhou, China). In addition, the relationships between digestion parameters (ADD and RGP) and amylose content across the two rice groups were evaluated using the Pearson's correlation analysis.

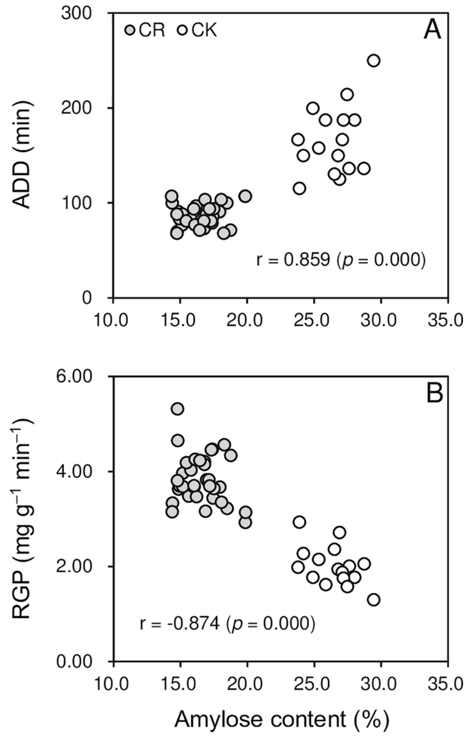

Fig. 4. Relationships between the active digestion duration (ADD) and amylose content (A) and between the rate of glucose production (RGP) within the ADD and amylose content (B) for the CR and CK groups of rice samples (see legend to Fig. 1 for sample details).

3. Results and discussion

The starch digestion of the cooked rice was well-fitted to the exponential association model, with coefficients of determination (r^2) of 0.985–0.998 and 0.990–0.999 for rice samples in the CR and CK groups, respectively (Fig. 3A). The fitting results showed that there was a large difference in the starch digestion of cooked rice among the tested samples, with a wide range of values of the model parameters (a and b) for rice samples in both the CR and CK groups. This result indicates variation in the starch digestion properties of rice.

More interestingly, the process and characteristics of starch digestion (except for TGP, $p = 0.267$) showed distinct differences between the rice samples in the CR and CK groups (Fig. 3A–D). In detail, ADD ranged from 68 to 107 min in rice samples in the CR group, with a mean of 87 min (Fig. 3B). In rice samples in the CK group, the minimum and maximum values of ADD were 115 and 250 min, respectively, with a mean of 166 min, which was approximately 90% longer than the ADD for rice samples in the CR group ($p = 0.000$). TGP ranged from 282 to 362 mg g^{-1} with a mean of 328 mg g^{-1}, and 274 to 354 mg g^{-1} with a mean of 321 mg g^{-1} in rice samples in the CR and CK groups, respectively (Fig. 3C). The minimum and maximum values of RGP in the CR group samples were 2.93 and 5.32 mg g^{-1} min^{-1}, respectively, with a mean of 3.84 mg g^{-1} min^{-1} (Fig. 3D). In the CK group samples, RGP ranged from 1.30 to 2.94 mg g^{-1} min^{-1}, with a mean of 2.01 mg g^{-1} min^{-1}, which was nearly 50% lower than that in rice samples in the CR group ($p = 0.000$). These results suggest that the rate of digestion of starch into glucose of cooked rice was higher in rice samples from the CR group than in samples from the CK group.

There have been reports showing that the rate of digestion of starch into glucose of cooked rice is closely related to amylose content (Fitzgerald et al., 2011; Frei et al., 2003; Hu et al., 2004). In general, the lower the amylose content, the higher rate of digestion of starch into glucose of cooked rice. In this study, we consistently observed a significant positive relationship between ADD and amylose content ($p = 0.000$; Fig. 4A), and a significant negative relationship between RGP and amylose content for rice samples in the CR and CK groups ($p = 0.000$; Fig. 4B). However, it has been well-documented that starch digestion properties of cooked rice are not only closely related to its amylose content but also affected by its other starch traits including starch gelatinization and retrogradation properties, the particle size of starch granule, the ratio of amylose to amylopectin, the crystallite structure of amylopectin, the starch resistance against enzymatic hydrolysis, and interactions of starch with other components (Huang and Hu, 2021). Therefore, additional investigations are required to focus on other starch traits in addition to amylose content to fully understand factors determining the starch digestion of low amylose rice. In addition, this study measured the starch digestion using an *in vitro* method; however, the results of *in vitro* methods may be different to those obtained using *in vivo* methods because of the difficulties in accurately simulating the highly complex physicochemical and physiological events occurring in human digestive tracts (Hur et al., 2011). Hence, it is necessary to perform *in vivo* studies to verify the results of this study.

Taken together, the findings of this *in vitro* study show that the development of low amylose rice in China can result in an acceleration in the rate of digestion of starch into glucose of cooked rice. Prior to this study, the risk of type 2 diabetes associated with white rice consumption has been documented in China (Hu, 2011; Hu et al., 2012; Ma et al., 2014), but no attention has been given to the potentially increased risk of type 2 diabetes resulting from the development of low amylose rice, which has received unprecedented interest and support in rice research and production in China in recent years (Liu et al., 2020; Yin et al., 2020; Zeng et al., 2019). We hope that the results of our study will serve to attract attention and help raise societal awareness of the health risks associated with this type of rice in China and other major rice-consuming countries. We also appeal to the government to immediately initiate an interdisciplinary collaboration between crop and food scientists and the public health sector to *in vivo* assess the potential risk of diabetes associated with the consumption of low amylose rice in China.

CRediT authorship contribution statement

Min Huang: Conceptualization, Formal analysis, Writing – original draft, Funding acquisition. **Liqin Hu:** Investigation. **Jiana Chen:** Investigation. **Fangbo Cao:** Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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