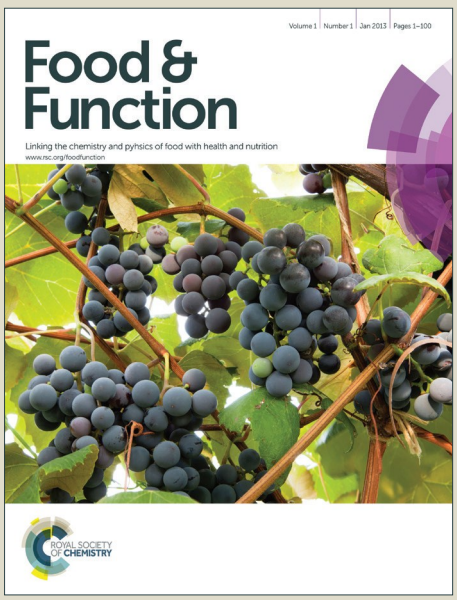
Food & Function

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/foodfunction

Food & Function

ARTICLE

In vitro starch digestibility and *in vivo* glycemic response of foxtail millet and its products

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Xin Ren,[a] Jing Chen,[a] Mohammad Mainuddin Molla,[a] Chao Wang,[a] Xianmin Diao[b] and Qun Shen*[a]

Foxtail millet, as the leading variety in arid and semiarid areas of Asia and Africa, can provide broad potential benefits to human health. However, its digestion properties are still not available. So in this study, the *in vitro* starch digestibilities and *in vivo* glycemic indices (GI) of foxtail millet and pure millet products were investigated. The results showed that starch digestibility of foxtail millet flour is obviously lower than that of wheat flour. However, deproteinization and heating significantly increased its rapidly digestible starch and decreased its slowly digestible starch and resistant starch. The GIs of pure millet products were in the following order: millet porridge (93.6 ± 11.3)> millet steamed bread (89.6 ± 8.8)> No. 1 millet pancake (75.0% millet flour and 25.0% extrusion flour, 83.0 ± 9.6)> No. 2 millet pancake (without extrusion flour, 76.3 ± 10.7)> cooked millet (64.4 ± 8.5). They were significantly positively correlated with the rapidly digestible starch (r = 0.959), degree of gelatinization (r = 0.967) and estimated glycemic index (r = 0.988). Both *in vitro* and *in vivo* tests suggested that boiling, steaming and extrusion enhanced the formation of digestible starch and subsequently increased the GI values. Additionally, the No. 1 millet pancake and cooked millet had a relatively gentle stimulation to *β*-cell. Therefore, foxtail millet, especially the cooked millet, may serve as a potential source of nutraceutical and functional food that could delay the development of type 2 diabetes.

Introduction

Type 2 diabetes has become a major health-threatening problem in many countries of the world. As it is, it has reached epidemic proportions, specifically, in China, up to 92.4 million people (9.7% of the general adult population) have been experiencing type 2 diabetes, and up to 148.2 million people (15.5%) have been experiencing prediabetes.[1] As is known, the quantity and quality of dietary carbohydrates played a critical role in the control of postprandial blood glucose.[2] Several studies have shown that slowly digested and absorbed carbohydrates were independently associated with the decreased risk of developing type 2 diabetes,[3-5] and several official dietary guidelines have recommended using the glycemic index (GI) for food choices.[6] Many factors may decrease the rate and extent of starch digestion and subsequently GI values, including the enzyme resistance of amylose-lipid complexes,[7] the encapsulation of protein matrix,[8, 9] and the processing method with low temperature, short time and insufficient water.[3, 10] Nowadays, people are able to produce low-GI foods, such as millet, pasta and foods containing modified starch, by controlling the ingredients and processing conditions.[4, 11]

Millet is a generic term including a range of small seeded cereals, such as pearl millet (*Pennisetum glaucum*), foxtail millet (*Setaria italica*), proso millet (*Panicum miliaceum*), finger millet (*Eleusine coracana*), and common millet (*Panicum miliaceum*). It has been used to produce porridge, wine, nutrition powder and several national products like *kunu*, *fura*, *upma* and *Laddu*.[12] Foxtail millet is the leading variety in China and it has been first domesticated and selected as grain food in the Yellow River basin as early as 8700 years ago.[13, 14] It is one of the most important drought-resistant crops and plays a critical role in food security in arid and semiarid areas of Asia and Africa.[12] It has been reported that foxtail millet can lower the risk of type 2 diabetes[15] and cardiovascular disease.[16] It has a high phytochemical content with antioxidative and antiproliferative activities.[17] Feeding of foxtail millet decreased the C-reactive protein and triacylglycerol levels in hyperlipidemic rats[18] and improved insulin sensitivity and cholesterol metabolism in genetically type 2 diabetic mice.[16] Additionally, both haematological and histological changes confirmed that foxtail millet bran oil was capable of attenuating ethanol-induced hepatic injury.[19] There has been growing interest in its nutritive value and potential health benefits in recent years, however, it remained not fully studied and utilized.[20] Starch, as a major component of foxtail millet, may determine the nutritional qualities and physiological properties of millet products. However, there are still no such reports regarding the starch digestion characteristics and glycemic responses of foxtail millet.

Therefore, the objectives of this study were (a) to evaluate the effects of lipid and protein on the contents of different starch fractions of foxtail millet in raw and cooked conditions with wheat flour as a positive control; (b) to determine the effects of different processing methods on the *in vitro* starch digestion characteristics, the degree of gelatinization (DG) and the estimated glycemic index

[a] National Engineering and Technology Research Center for Fruits and Vegetables, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China. E-mail: shengun@cau.edu.cn; Tel: 86-10-62737524; Fax: 86-10-62737524
[b] National Key Facility for Crop Gene Resources and Genetic Improvement, Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081, China.

Food & Function Accepted Manuscript

Please do not adjust margins

(eGI); and (c) to monitor the blood glucose and insulin responses of ten health adults after the ingestion of five pure foxtail millet products.

Materials and methods

Foxtail millet and enzymes

Foxtail millet (*Setaria italica*) was purchased from Jinguzi Company (Tianjin, China). It was milled by a WF-20B pulverizer (Keyi Machinery, Nanjing, China) and ground through a 0.2 mm sieve, then stored at 4 °C. Amyloglucosidase (catalogue No. 10113), invertase (catalogue No. I4504), pancreatin (catalogue No. P7545) and pepsin (catalogue No. P7000) were purchased from Sigma-Aldrich (St Louis, MO, U.S.).

Samples preparation

Extrusion powder. The pre-prepared foxtail millet flour was extruded via an SLG30-IV twin-screw extruder (Saibainuo Technologies, Jinan, China). The barrel temperatures were 60, 90, 120 and 175 °C, respectively, with moisture contents being 16.0 (w/w, wet basis) and screw speed being 30 rpm. Samples were collected after the extruder reached a steady state. After extrusion, the puffing materials were milled and ground through a 0.2 mm sieve again. Moreover, the contents of moisture[21] and available carbohydrates[22] were determined.

Pure foxtail mllet products. Millet steamed bread (MSB) was steamed with 75.0% millet flour and 25.0% extrusion flour (w/w, dry basis). No.1 millet pancake (MP-1), also together with 75.0% millet flour and 25.0% extrusion flour (w/w, dry basis), was baked in a pan. No.2 millet pancake (MP-2) was also baked in a pan without extrusion flour. Another two products were cooked millet with a millet: water ratio of 1:1.5 and millet porridge with a millet: water ratio of 1:9. The freshly prepared products were wet-ground for 3 seconds by a JYL-C012 machine (Joyoung, Hangzhou, China) and subjected to the *in vitro* test in a form that resembles the food "as eaten".[2]

Defatted or/and deproteined millet flour. The foxtail millet flour was placed into several flasks, followed by adding n-hexane (1:5 w/v) thereto, stirring and mixing well for defatting, sealing the flasks with tin foil paper and placing them in a water bath at 45 °C for continue stirring at 160 rpm for 120 min, vacuum-filtering by a vacuum suction pump to collect residues, and drying the obtained residues via air stream to obtain the defatted millet flour. In addition, the foxtail millet flour and defatted millet flour were placed into several flasks, respectively, followed by adding freshly-prepared pepsin solution (5.0 g/L pepsin in 0.05 mol/L HCl), stirring and mixing well for deproteining, placing them in a water bath at 37 °C for continue stirring at 160 rpm for 30 min, then centrifuging at 1500*g* for 10 min to collect residues, and freeze-drying the obtained residues to obtain the deproteined millet flour and millet starch. The contents of protein and lipid of each samples were listed in in Table 1.Finally, the foxtail millet flour and the flour with lipid removed, protein removed, or both lipid and protein removed were dispersed in 5.0 mL of water and heated in a boiling water bath for 20 min to obtain the cooked samples. The wheat flour (Jinshahe Flour Manufacturing, Hebei, China) were used as a positive control.

In vitro starch digestibility

The samples were analyzed for the *in vitro* starch digestion based on Englyst et al.[2, 23] with some modifications. The samples (containing about 0.5 g of starch) were dispersed in 25.0 mL of acetate buffer (0.1 M, pH 5.2) in 50-mL centrifuge tubes with 2 glass balls. After vortex-mixed vigorously, the tubes were placed into a boiling water bath for 30 min and cooled to 37 °C, then invertase (3000 U/mL, 0.3 mL) was added, vortex-mixed and incubated at 37 °C for 30 min. Finally 0.2 mL of each sample was added into 4 mL of absolute ethanol and mixed well to obtain the free glucose (FG) portion.

As above, another samples were dispersed in 10.0 mL of freshly-prepared pepsin solution (5.0 g/L pepsin and 5.0 g/L guar gum in 0.05 mol/L HCl, 5 glass balls), placed in a water bath at 37 °C for 30 min, and then added with 10 mL of acetate buffer (0.1 M, pH 5.5, 37 °C). 5.0 mL of enzyme mixture was added to initiate starch digestion wherein the enzyme mixture was prepared by dispersing 3.0 g of pancreatin in 20.0 mL of water via a magnetic stirrer for 10 min, then centrifuging at 1500*g* for 10 min to obtain pancreatin supernatant (15.0 mL), and adding 0.75 mL of amyloglucosidase (1200 U/mL) and 1 mL of invertase (3000 U/mL) thereto. The samples were digested at 37 °C for 2 h under horizontal shaking at 160 rpm. After exactly 20 and 120 min of digestion, 0.2 mL of each sample was added into 4.0 mL of absolute ethanol and mixed well to obtain the glucose portion for 20 min (G_{20}) and 120 min (G_{120}).

After 0.2 ml of G_{120} samples has been collected, the tubes were vortex-mixed vigorously. After boiling-water incubation for 30 min, the contents were cooled to 0 °C and mixed with 10.0 mL of 7.0 mol/L potassium hydroxide. After ice-water incubation for 30 min, 0.2 mL of each sample was added to 1.0 mL of 1.0 mol/L acetic acid containing 40.0 μL of amyloglucosidase (100.0 U/mL), followed by placing in 70 °C water bath for 30 min and boiling-water bath for 10 min, then cooling to room temperature and adding 20.0 mL of water to obtain the total glucose portion (TG).

All above collected samples (FG, G_{20}, G_{120} and TG) were centrifuged at 1500*g* for 5 min. The glucose content in the supernatant was measured using the glucose oxidase-peroxidase method by a GOD-POD diagnostic kit (Applygen Technologies, Beijing, China). The OD values (x-axis) were measured by Thermo Scientific Multiskan GO (Thermo Fisher Scientific, MA, U.S.). Standard glucose solutions with concentrations of 125.0, 250.0, 500.0, 1000.0 and 2000.0 μM/L each were subjected to the same tests, respectively, at the same time to thereby obtain a standard curve ($y = 4526x - 21.7$, $R^2 = 0.9998$).

Degree of gelatinization (DG)

An enzyme method[24] for detecting DG was applied in this study. In short, 50.0 mg of freshly wet-ground sample was accurately weighed into a 10.0 mL centrifuge tube, together with 1.0 mL of amyloglucosidase (50.0 U/mL) and 4.0 mL of acetate buffer (0.1 M, pH 4.75). The contents were vortex-mixed and the tubes were placed into a 37 °C water bath for 30 min under horizontal shaking at 160 rpm. Then 0.2 mL of each sample was added into 4.0 mL of absolute ethanol, mixed well and centrifuged at 1500*g* for 5 min. The glucose

Food & Function Accepted Manuscript

 This journal is © The Royal Society of Chemistry 20xx

Please do not adjust margins

content in the supernatant was measured as described above. Another same samples were autoclaved at 121 °C for 30 min for full gelatinization and thereafter subjected to the same procedures. The DG was defined as the glucose content of per gram of original sample, expressed as a percentage of that for per gram of fully gelatinized sample.

Estimated glycemic index (eGI)

The kinetics of the *in vitro* starch digestibility and the eGI were calculated on the basis of glucose measurement at different times (20, 40, 60, 80, 100, 120 and 180 min) during above starch hydrolysis. A first order equation [$C = C_\infty(1 - e^{-kt})$] was applied,[25] where C, C_∞ and k represented the percentage of starch hydrolyzed at time t (min), the maximum hydrolysis extent and the kinetic constant, respectively. The hydrolysis index (HI) was obtained based on the relationship between area under hydrolysis curve (AUC) for millet product and the AUC for a reference food (fresh white bread). The eGI (bread = 100) was calculated using the equation $\mathrm{eGI} = 39.71 + 0.549\mathrm{HI}$ and it was multiplied by 0.7 to obtain the eGI value with glucose as the reference food (glucose = 100).[6]

***In vivo* glycemic response**

The *in vivo* glycemic response of five freshly-prepared pure foxtail millet products were determined in ten healthy subjects (three males and seven females, mean age = 26.0, mean BMI = 20.8 kg/m²).[26, 27] The consumption amount of test foods which can provide 50.0 g of available carbohydrate was listed in Table 2. Each subject consumed the test foods and standard glucose solution in a random order on separate mornings (3 days apart) after 10-12 h of overnight fasting. For collection of venous blood samples, an intravenous catheter (BD Insyte 20 GA × 1.16 IN, 1.1 × 30 mm; Becton Dickinson Infusion Therapy Systems,) was applied in this study. After collecting the 2 mL of fasting blood sample, subjects ate test meal at a comfortable speed within 15 min and then 2 mL of further blood samples were collected at 15, 30, 45, 60, 90 and 120 min, respectively. Test meals were provided together with 200.0 mL of water and standard glucose solution was measured twice. Blood samples were collected into tubes and immediately separated by centrifugation and stored at -80 °C for analysis. Plasma glucose was measured using the Roche P800 analyzer (Roche-Diagnostics, Basel, Switzerland) by enzymatic determination. Plasma insulin was measured with an immunoluminometric assay using the Siemens ADVIA Centaur XP analyser (Siemens Healthcare Diagnostics, Washington, U.S.). Ethical permission for this study was obtained from the Biomedicine Ethical Committee of Peking University, and the written informed consent was given to subjects.

Statistical analysis

According to Englyst et al.,[2] from the data of *in vitro* starch digestion, the contents of different starch fractions: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS), as well as the contents of different available glucose fractions: rapidly available glucose (RAG) and slowly available glucose (SAG) in dry basis were calculated as follows:

$$\mathrm{RAG} = G_{20} \quad (1)$$

$$\mathrm{SAG} = G_{120} - G_{20} \quad (2)$$

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

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

$$\text{Total starch} = (TG - FG) \times 0.9 \quad (5)$$

$$\mathrm{RS} = (TG - G_{120}) \times 0.9 \quad (6)$$

Results in this study were expressed as a percentage of total starch or total available glucose.[28] The AUCs of blood glucose and insulin were calculated according to the trapezoidal rule in geometry, ignoring any area beneath the fasting level. The GI and insulin index (II) for the test foods were calculated by the average of individual values.[27]

In all cases, at least three replicates were performed for each analysis. All analyses were performed using SPSS Statistics 17.0 (SPSS Inc., Chicago, IL, U.S.). Data for *in vitro* digestibility was presented as the mean ± standard deviation (SD) and for *in vivo* digestibility (GI and II) was presented as the mean ± standard error of the mean (SEM). One-Way ANOVA was used followed by Tukey's test and a p value under 0.05 was considered to indicate significance.

Results and discussion

Effects of components and heating on the *in vitro* starch digestibility

Starch, protein and lipid are three major components in cereal-based foods. Interactions among them play an important role in starch digestibility in human small intestine and therefore influence further blood glucose response.[11] The effects of lipid and protein on *in vitro* starch digestion characteristics of foxtail millet were investigated in this study. As shown in Figure 1, the different starch fractions (RDS, SDS and RS) were determined in millet flour, defatted millet flour, deproteined millet flour, and millet starch, respectively, with wheat flour as a positive control. The content of RDS in raw millet flour was the lowest (37.7 ± 3.2, mean ± SD). By comparison, it can be seen that the content of RDS increased slightly in defatted millet flour (40.1 ± 1.6) but significantly in deproteined millet flour and millet starch (50.9 ± 2.9 and 53.4 ± 1.3, respectively). Conversely, both the content of SDS and RS in raw millet flour was the highest, companying with that of defatted millet flour being slightly decreased, and that of deproteined millet flour and millet starch being drastically decreased respectively. These results were in consistence with those obtained in previous studies, i.e. fats formed complexes with amylose and proteins blocked enzyme adsorption sites on the surface of starch granule.[7, 9, 29] The amylose-lipid complexes had enzymatic resistance which increased with increasing lipid chain length, amylose degree of polymerization and complexation temperature.[7] Additionally, the hydrophobicity of lipids also significantly affect the starch digestibility. Proteins reduced starch granule surface accessibility and therefore influenced the enzyme susceptibility. More specifically, Protein fractions, such as albumin, globulin and glutenin, were glued

Food & Function Accepted Manuscript

Please do not adjust margins

into a matrix surrounding starch granules acted as a barrier against starch digestion.[11] This phenomenon has been confirmed by adding protease to corn flour or removing gluten from wheat flour, both of which resulted in a significant enhancement of *in vitro* starch digestibility.[11] Many other studies also reported the presence of protein barrier, such as sorghum kafirin[29] and pasta.[9]

In addition, it could be suggested that, under the present study condition, the surface protein has a greater effect than surface lipid on *in vitro* starch digestibility of foxtail millet. This result, however, was inconsistent with those found by Annor et al.[30] in kodo millet flour, possibly due to the different interactive model among different free fatty acids, proteins and starch.[7]

After cooking, the changes among different samples were almost the same (Figure 1). In detail, the RDS of all samples increased significantly, companying with a considerable decrease of SDS and RS. This phenomenon was the most obvious in wheat flour, in which the RDS content increased from 46.6 ± 3.0 to 95.5 ± 0.7. In general, starch gelatinization was characterized by physical and chemical changes, such as swelling, rupturing and the disruption of crystalline structure. From the result of this study, it can be further suggested that in terms of biochemical change, the starch gelatinization was such a process that SDS and RS turned into RDS. Martine et al.[31] had reported that the starch granule swelling behavior can be identified to three classes, the data from cooked samples showed that foxtail millet starch should be classified as second class: slow swelling, which can be converted to rapid swelling by extraction of surface proteins and lipids.

More interestingly, the starch digestibility of millet flour was significantly lower than that of wheat flour both in raw and cooked conditions. The RDS content of cooked millet flour was just 61.0% of that of cooked wheat flour (Figure 1). That is, the millet flour had relatively low enzyme susceptibility and resisted to enzymatic hydrolysis in some extent. This result was consistent with those observed in kodo millet, finger millet and barnyard miller, but not consistent with that observed in proso millet.[20] After both lipid and protein were removed from millet flour, the digestibility was slightly higher than that of wheat flour in raw materials and almost the same as in cooked ones, which indicated once more that the presence of protein and lipid decreased the starch digestion rate of foxtail millet.

Effects of different processing methods on the *in vitro* starch digestibility and estimated glycemic index

There were many factors contributing to the *in vitro* starch digestibility, such as amylose content, cultivar, partial size, processing and storage condition.[11, 27] Among all these, food processing was the major determinant of starch gelatinization, digestion and absorption, and eventually influenced the final postprandial glycemic response.[3, 32] There were evidences that the starch fractions of finger millet (ragi)[10] and the glycemic index of potato[3] varied significantly depending on the different cooking methods. In the present study, the effects of different processing methods (steaming, pan-baking, cooking and boiling) on starch digestion and gelatinization properties of foxtail millet were investigated (Table 3). The DG of millet porridge was the highest (93.5 ± 0.1, mean ± SD), followed by MSB (86.5 ± 0.2) and two MP (70.2 ± 1.9 for MP-1 and 67.7 ± 1.1 for MP-2 respectively), and the DG of cooked millet was the lowest (55.5 ± 2.9). This trend was also observed in the contents of RAG and RDS, suggesting that the DG of specific food strongly affects its digestibility.[24] From the point of available glucose, the RAG content ranged from 51.3 ± 5.8 to 65.1 ± 5.6 after various cooking methods, that is, the RAG always was the dominant component in five pure millet products. From the point of starch, millet porridge showed the highest RDS content, which may support the opinions of Englyst[23] who reported that the RDS content of boiled millet was about 56.0%. Except for millet porridge, there was no significant difference between the contents of RDS and SDS. However, the RS content showed a wide variation from 8.8 ± 3.9 to 24.9 ± 3.6, and the highest RS content was observed in cooked millet which was corresponding to the lowest DG. RS is considered as a source of dietary fiber and can provide a number of beneficial effects. The decrease in digestible starch and increase in RS content of cooked millet would be expected to improve human health.

The kinetics of *in vitro* starch digestibility and eGI of pure foxtail millet products were listed in Table 4. The maximum hydrolysis extent, or equilibrium concentration, C_∞, ranged between 76.5 ± 1.6 and 92.1 ± 2.0. These results were obviously higher than those of legumes ranged from 33.1 to 43.1,[33] but lower than those of gluten-free breads with an average of 96.5.[34] The kinetic constant, *k*, which reflects the rate of hydrolysis in the early stage, ranged between 0.030 ± 0.002 and 0.040 ± 0.001. The *k* was the lowest in MP-2, which was almost the same as MP-1. More interestingly, the trend of C_∞ and *k* were not fully consistent with each other. The *k* of cooked millet was higher than that of pancake but its C_∞ was much lower. That is, in terms of cooked millet, although its hydrolysis rate was faster in the early stage, its equilibrium hydrolysis extent was smaller. The eGI, either white bread or glucose used as the reference food, followed the order: Millet porridge > MSB >MP-1 >MP-2 > Cooked millet. This trend was consistent with the results of DG and RDS. According to the above discussion, it can be concluded that the eGI was a result of joint effect of C_∞ and *k*, and reflected the starch digestibility more succinctly in this portion.

Roasting, autoclaving and pressure-cooking enhanced the formation of RDS in finger millet;[10] boiling, mashing and extrusion-cooking contributed to significant increase of digestible starch in potato.[3] In the present study, although the raw materials of MSB and MP-1 were exactly the same, the RAG, RDS, DG and eGI of MSB were always higher than those of MP-1, which represented that steaming enhanced the formation of digestible starch. Although two millet pancakes were obtained by same processing method, the MP-1 which had 25.0% extrusion flour, exhibited higher digestibility during the whole hydrolyzation. Nowadays, extrusion cooking has been widely used for the production of precooked flours, snack foods, and breakfast cereals. During the extrusion process, high temperatures, pressures and shear forces destroyed the starch granular structure, thereby decreased the crystallinity and led to partial depolymerisation, and therefore increased its gelatinization extent and enzymes availability. Many researchers have reported that the extrusion cooking significantly increased the *in vitro* starch digestibility of potatoes, beans, corns and barleys.[3, 11] Our results

Food & Function Accepted Manuscript

 This journal is © The Royal Society of Chemistry 20xx

Please do not adjust margins

confirmed this phenomenon in foxtail millet. The addition amount of water during processing was also an important factor determining the DG and starch digestibility.[4, 11] When starch-based materials were heated in excess water, such as millet porridge, the water molecules linked to the exposed hydroxyl groups of amylose and amylopectin, which caused an increase in granule swelling and complete gelatinization.[11] Thus the cooked millet, when compared with millet porridge, had a smaller RDS, DG and eGI for the quite low water content. This phenomenon was also observed in biscuits[32] and fried potatoes[3]. In addition, milling can increase the surface area and subsequent enzyme susceptibility of starch granule. Therefore, although the water contents of MSB and MP were lower than that of cooked millet, their DG and starch digestibility was still much higher.

Effects of different processing methods on the *in vivo* starch digestibility

The GI, which was first introduced by Jenkins et al.[26] in 1981, has been widely accepted as a golden standard of carbohydrate classification and primary guidance of food choice.[5, 6] However, it is still controversial whether the *in vitro* digestion characteristics can reflect the glycemic responses accurately.[6, 35] And only a few foods have been subjected to both *in vitro* and *in vivo* testing for comparison.[3] So in order to give a comprehensive evaluation of the effects of different processing methods on starch digestibility of foxtail millet, we further investigated the blood glucose and insulin responses after ingestion of pure millet products (Figure 2). The peak time and concentration were two main factors of blood glucose curve. From the data (figure 2A), it can be observed that the peak concentration of millet porridge was the highest (8.0 ± 0.4, mean ± SD), even higher than that of the standard glucose solution (7.4 ± 0.2), followed by MP-1 (7.3 ± 0.3), MSB (7.1 ± 0.6), MP-2 (6.6 ± 0.2) and cooked millet (6.4 ± 0.2). The peak time of millet porridge, MP-1 and MSB was 45 min, while that of others was 30 min. The blood glucose concentration of cooked millet, followed by MP-2, was always apparently lower than that of standard glucose solution within 2 h. In detail, the maximum increase of blood glucose in cooked millet just was 63.4% of that in standard glucose solution and 55.7% of that in millet porridge. Moreover, only the blood glucose level of MP-2 at 120 min was lower than fasting level. From the blood insulin reaction curves (Figure 2B), it can be observed that when compared with standard glucose solution (555.0 ± 107.8), the peak concentration of millet porridge (608.1 ± 97.5) was slightly higher and that of MSB (551.5 ± 137.0) and MP-2 (522.0 ± 140.0) were slightly lower, and the peak concentration of MP-1 (383.0 ± 47.9) and cooked millet (288.8 ± 64.2) were the lowest. The peak time of cooked millet was 30 min, while that of others was 45 min. Similar to the blood glucose curve, the insulin concentration of cooked millet, followed by MP-1, was always apparently lower than that of standard glucose solution within 2 h. The maximum increase of blood insulin in cooked millet just was 49.1% of that in standard glucose solution and 43.3% of that in millet porridge.

The GI, II and II to GI ratio (II / GI) were calculated (Table 4). The results showed that the GI of millet porridge was the highest, followed by MSB, MP-1, MP-2 and cooked millet. These findings were similar to the international tables which reported that the GI of millet flour porridge (Kenya) was 107,[6] but apparently higher than the results provided by Yang et al. who found that the GI of millet porridge was just 61.5 ± 9.[36] Many factors (such as food ingredients and processing methods) may result in the differences in starch digestibility and subsequently GI values for apparently similar foods.[6, 11, 27] For instance, the published GI values of potatoes and potato-products varied from 23 to 144.[3] The above difference can be attributed to the inherent botanical differences and methodological factors, especially the measurement of available carbohydrate content. Among the five pure foxtail millet products, only cooked millet was classified as medium-GI food (from 55 to 70 on the glucose reference scale). MP-2, MP-1, MSB and millet porridge were all available in high-GI forms (70 or greater). Even so, the GI of foxtail millet was apparently lower than those of wheat and rice[6], and this result has been confirmed by above investigation which showed the starch digestibility of foxtail millet flour was significantly lower than wheat flour no matter in raw materials or cooked ones.

Furthermore, The GI of pure millet produces was significant positively correlated with DG (r = 0.967, p = 0.007), RDS (r = 0.959, p = 0.01) and eGI (r = 0.988, p = 0.002). But no significantly positive relationship between GI and RAG was observed. This may be due to different forms of raw materials: flour and grain. To verify this hypothesis, millet products were sorted for comparison based on their material forms and the correlationship turned into apparent. Our results suggested that to some extent the *in vitro* starch digestion was a reliable index of the *in vivo* postprandial glycemic responses for a certain kind of food. But for the complexity of food matrix and gastrointestinal system, different kind of food may be suitable for different prediction. Therefore, more widely and concretely work needed to be done before the *in vitro* results of a specific kind of food can be used in clinical applications or epidemiologic research.

Considering the fact that insulin resistance is a key feature of type 2 diabetes and metabolic syndrome, another objective of the present study was to evaluate the effect of specific food on blood insulin response. The II of five pure foxtail millet products followed the order: MSB > Millet porridge > MP-2 > MP-1 > Cooked millet. Just based on the fact that the insulin/glucose ratio may be used to evaluate *β*-cell response,[37, 38] the II/GI was defined to evaluate the insulin demand for a specific food (Table 4). The II/GI of MSB and the II/GI of MP-2 were larger than 1.0, which indicated that ingesting MSB and MP-2 may induce a strong stimulation to *β*-cell. That is, quite an amount of insulins was needed after ingestion of MSB and MP-2. On the contrary, the II/GI of MP-1 and the II/GI of cooked millet was smaller than 1.0, so after ingestion of such foods, there was no need for *β*-cells to secrete too much insulin, the blood glucose can be maintained at a stable level. Coincidentally, the insulin AUC/glucose AUC, a similar concept to II/GI, has been used by Holt et al.,[39] who have found that the AUC ratio of white pasta was more than twice of that of brown pasta and the protein-rich foods stimulated a large amount of insulin secretion relative to their glycemic responses. In conclusion, the cooked millet was the most suitable pure foxtail millet product for type 2 diabetics.

Food & Function Accepted Manuscript

Conclusions

Blood glucose and insulin were essential for the health of both normal and diabetic subjects. Several prospective epidemiological studies have shown that diet, especially starch-based food, was crucial for maintaining homeostasis of blood glucose and insulin. Interestingly, results from this study confirmed that foxtail millet, as a kind of functional food, had a quite low GI value and a relatively gentle stimulation to β-cell. Moreover, different processing methods had a great influence on the digestibility and glycemic responses of foxtail millet, which suggested that in daily life, it was necessary for man to select appropriate processing method according to the healthy condition himself/herself. Additionally, there will be necessary to carry out further researches about diet intervention with foxtail millet among pre-diabetics or diabetics. The investigation of hypoglycemic effect of foxtail millet will be beneficial to promote the development of millet industry and to popularize the millet-based foods.

Abbreviations used

MSB, millet steamed bread; MP-1, No.1 millet pancake (75.0% millet flour and 25.0% extrusion flour); MP-2, No.2 millet pancake (without extrusion flour); RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch; RAG, rapidly avaliable glucose; SAG, slowly avaliable glucose; DG, degree of gelatinization; eGI, estimated glycemic index; GI, glycemic index; II, insulin index

Acknowledgements

The authors thank Chang Shu and Zenglong Chen for improving the language and providing valuable comments on this manuscript. This research was supported by the China Agriculture Research System (CARS-07-12.5-A17).

References

1 W. Yang, J. Lu, J. Weng, W. Jia, L. Ji, J. Xiao, Z. Shan, J. Liu, H. Tian and Q. Ji, *New Engl. J. Med.*, 2010, **362**, 1090-1101.

2 K. N. Englyst, H. N. Englyst, G. J. Hudson, T. J. Cole and J. H. Cummings, *Am. J. Clin. Nutr.*, 1999, **69**, 448-454.

3 B. Nayak, J. D. J. Berrios and J. Tang, *Food Res. Int.*, 2014, **56**, 35-46.

4 U. Lehmann and F. Robin, *Trends Food Sci. Tech*, 2007, **18**, 346-355.

5 B. Jennie, S. Hayne, P. Petocz and C. Stephen, *Diabetes. Care*, 2003, **26**, 2261-2267.

6 K. Foster-Powell, S. H. Holt and J. C. Brand-Miller, *Am. J. Clin. Nutr.*, 2002, **76**, 5-56.

7 G. G. Gelders, J. P. Duyck, H. Goesaert and J. A. Delcour, *Carbohyd. Polym.*, 2005, **60**, 379-389.

8 L. I. Ezeogu, K. G. Duodu, M. N. Emmambux and J. R. Taylor, *Cereal Chem.*, 2008, **85**, 397-402.

9 E. H.-J. Kim, J. R. Petrie, L. Motoi, M. P. Morgenstern, K. H. Sutton, S. Mishra and L. D. Simmons, *Food Biophys.*, 2008, **3**, 229-234.

10 S. Roopa and K. Premavalli, *Food Chem.*, 2008, **106**, 875-882.

11 J. Singh, A. Dartois and L. Kaur, *Trends Food Sci. Tech*, 2010, **21**, 168-180.

12 A. S. Saleh, Q. Zhang, J. Chen and Q. Shen, *Compr. Rev. Food Sci. F.*, 2013, **12**, 281-295.

13 H. Lu, J. Zhang, K.-b. Liu, N. Wu, Y. Li, K. Zhou, M. Ye, T. Zhang, H. Zhang and X. Yang, *P. Natl. Acad. Sci. Usa.*, 2009, **106**, 7367-7372.

14 C. Wang, G. Jia, H. Zhi, Z. Niu, Y. Chai, W. Li, Y. Wang, H. Li, P. Lu and B. Zhao, *G3: Genes| Genom.| Genet.*, 2012, **2**, 769-777.

15 S. Li and X. Luo, *Compendium of Materia Medica:(Bencao Gangmu)*, Foreign Languages Press, 2003.

16 Y.-Y. Choi, K. Osada, Y. Ito, T. Nagasawa, M.-R. Choi and N. Nishizawa, *Biosci. Biotech. Bioch*, 2005, **69**, 31-37.

17 L. Z. Zhang and R. H. Liu, *Food Chem.*, 2015, **174**, 495 501.

18 S. H. Lee, I.-M. Chung, Y.-S. Cha and Y. Park, *Nutr. Res.*, 2010, **30**, 290-296.

19 M. Pang, S. He, L. Wang, X. Cao, L. Cao and S. Jiang, *Food Funct.*, 2014, **5**, 1763-1770.

20 F. Zhu, *Food Res. Int.*, 2014, **64**, 200-211.

21 C. AACC, *Methods*, 2000, **54**, 21.

22 J. Frias, C. Vidal-Valverde, C. Sotomayor, C. Diaz-Pollan and G. Urbano, *Eur. Food Res. Technol*, 2000, **210**, 340-345.

23 H. N. Englyst, S. Kingman and J. Cummings, *Eur. J. Clin. Nutr.*, 1992, **46**, S33-50.

24 K. Liu and J. Han, *J. Agr. Food Chem.*, 2012, **60**, 4212 4221.

25 I. Goñi, A. Garcia-Alonso and F. Saura-Calixto, *Nutr. Res.*, 1997, **17**, 427-437.

26 D. Jenkins, T. Wolever, R. H. Taylor, H. Barker, H. Fielden, J. M. Baldwin, A. C. Bowling, H. C. Newman, A. L. Jenkins and D. V. Goff, *Am. J. Clin. Nutr.*, 1981, **34**, 362-366.

27 F. Brouns, I. Bjorck, K. Frayn, A. Gibbs, V. Lang, G. Slama and T. Wolever, *Nutr. Res. Rev.*, 2005, **18**, 145-171.

28 L. R. Brewer, L. Cai and Y.-C. Shi, *J. Agr. Food Chem.*, 2012, **60**, 4379-4387.

29 P. Belton, I. Delgadillo, N. Halford and P. Shewry, *J. Cereal Sci.*, 2006, **44**, 272-286.

30 G. A. Annor, M. Marcone, E. Bertoft and K. Seetharaman, *Cereal Chem.*, 2013, **90**, 211-217.

31 M. R. Debet and M. J. Gidley, *Carbohyd. Polym.*, 2006, **64**, 452-465.

32 K. N. Englyst, S. Vinoy, H. N. Englyst and V. Lang, *Brit. J. Nutr.*, 2003, **89**, 329-339.

33 H.-J. Chung, Q. Liu, R. Hoover, T. D. Warkentin and B. Vandenberg, *Food Chem.*, 2008, **111**, 316-321.

34 E. de la Hera, C. M. Rosell and M. Gomez, *Food Chem.*, 2014, **151**, 526-531.

35 M. Minekus, M. Alminger, P. Alvito, S. Ballance, T.

Food & Function Accepted Manuscript

 This journal is © The Royal Society of Chemistry 20xx

Please do not adjust margins

Bohn, C. Bourlieu, F. Carrière, R. Boutrou, M. Corredig and D. Dupont, *Food Funct.*, 2014, **5**, 1113-1124.

36 Y.-X. Yang, H.-W. Wang, H.-M. Cui, Y. Wang, L.-D. Yu, S.-X. Xiang and S.-Y. Zhou, *World journal of gastroenterology: WJG*, 2006, **12**, 3430-3433.

37 R. A. DeFronzo, J. D. Tobin and R. Andres, *Am. J. Physiol-gastr. L*, 1979, **237**, G214-G223.

38 R. L. Hanson, R. E. Pratley, C. Bogardus, K. V. Narayan, J. M. Roumain, G. Imperatore, A. Fagot-Campagna, D. J. Pettitt, P. H. Bennett and W. C. Knowler, *Am. J. Epidemiol.*, 2000, **151**, 190-198.

39 S. Holt, J. Miller and P. Petocz, *Am. J. Clin. Nutr.*, 1997, **66**, 1264-1276.

Food & Function Accepted Manuscript

Please do not adjust margins

Food & Function

ARTICLE

Tables

Table 1. The content/ remove ratio of protein and lipid of each samples

Sample	protein (g/100g)	Lipid (g/100g)	remove ratio of protein (%)	remove ratio of lipid (%)
Millet flour	9.76 ± 0.03 d	1.85 ± 0.012 d	-	-
Defatted millet flour	9.41 ± 0.03 c	0.29 ± 0.009 b	3.61 ± 0.28 a	84.40 ± 0.49 b
Deproteined millet flour	2.54 ± 0.003 a	0.89 ± 0.004 c	73.99 ± 0.03 b	51.98 ± 0.24 a
Millet starch	2.60 ± 0.001 b	0.22 ± 0.005 a	73.33 ± 0.01 b	88.10 ± 0.25 c

Values (mean ± SD) followed by a different letter in each column were significantly different ($P < 0.05$).

Table 2. The consumption amount of pure foxtail millet products and standard glucose solution‡

Variety	Water Content (%)	Available Carbohydrate Content (%)	Available Carbohydrate Amount (g)	Consumption Amount (g)
MSB	42.0	50.1	50.0	100.0
MP-1	59.0	35.4	50.0	141.0
MP-2	52.0	41.2	50.0	121.0
Cooked Millet	65.4	29.7	50.0	169.0
Millet Porridge	89.4	9.1	50.0	550.0
Glucose Solution	80.0	20.0	50.0	250.0

‡MSB, millet steamed bread; MP-1, No. 1 millet pancake (75.0% millet flour and 25.0% extrusion flour); MP-2, No. 2 millet pancake (without extrusion flour).

Table 3. The effect of different processing methods on starch digestion and gelatinization properties in foxtail millet‡

Sample	Starch Fraction			Available Glucose		DG %
	RDS %	SDS %	RS %	RAG %	SAG %	
MSB	46.3 ± 6.7 ab	44.9 ± 4.6 a	8.8 ± 3.9 a	55.4 ± 6.6 a	44.7 ± 6.6 a	86.5 ± 0.2 c
MP-1	43.0 ± 1.3 abc	46.3 ± 5.9 a	10.7 ± 5.0 a	53.0 ± 3.9 a	47.0 ± 3.9 a	70.2 ± 1.9 b
MP-2	39.1 ± 2.3 bc	45.0 ± 6.3 a	15.9 ± 4.3 ab	51.3 ± 5.8 a	48.7 ± 5.8 a	67.7 ± 1.1 b
Cooked Millet	36.9 ± 1.4 c	38.3 ± 2.2 ab	24.9 ± 3.6 c	52.9 ± 0.6 a	47.1 ± 0.6 a	55.5 ± 2.9 a
Millet Porridge	50.7 ± 4.2 a	40.5 ± 3.2 ab	8.8 ± 2.7 a	65.1 ± 5.6 b	34.9 ± 5.6 b	93.5 ± 0.1 d

‡MSB, millet steamed bread; MP-1, No. 1 millet pancake (75.0% millet flour and 25.0% extrusion flour); MP-2, No. 2 millet pancake (without extrusion flour); RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch; RAG, rapidly avaliable glucose; SAG, slowly avaliable glucose; DG, degree of gelatinization. Values (mean ± SD) followed by a different letter in each column were significantly different ($P < 0.05$).

Food & Function Accepted Manuscript

Table 4. In vitro hydrolysis kinetics and *in vivo* glycemic indices of pure foxtail millet products‡

Sample	C_∞	K	eGI (Bread = 100)	eGI (Glucose = 100)	GI	II	II/GI
MSB	90.8 ± 1.8 b	0.036 ± 0.001 b	86.3 ± 1.1 c	60.4 ± 0.8 c	89.6 ± 8.8 ab	109.3 ± 11.5 c	1.2 ± 0.2 b
MP-1	92.1 ± 2.0 b	0.031 ± 0.001 a	84.9 ± 0.7 c	59.4 ± 0.5 c	83.0 ± 9.6 ab	65.0 ± 4.0 ab	0.8 ± 0.1 a
MP-2	86.5 ± 3.8 b	0.030 ± 0.002 a	81.7 ± 1.3 b	57.2 ± 0.9 b	76.2 ± 10.7 ab	84.5 ± 14.4 bc	1.1 ±0.1 ab
Cooked Millet	76.5 ± 1.6 a	0.033 ± 0.001 ab	77.6 ± 0.6 a	54.3 ± 0.4 a	64.4 ± 8.5 a	49.8 ± 7.6 a	0.8 ± 0.1 a
Millet Porridge	91.8 ± 1.7 b	0.040 ± 0.001 c	86.8 ± 0.6 c	60.7 ± 0.5 c	93.6 ± 11.3 b	85.8 ± 9.8 bc	0.9 ± 0.1 ab

‡MSB, millet steamed bread; MP-1, No. 1 millet pancake (75.0% millet flour and 25.0% extrusion flour); MP-2, No. 2 millet pancake (without extrusion flour); C_∞, maximum hydrolysis extent; k, kinetic constant; HI, hydrolysis index; Egi, estimated glycemic index; GI, glycemic index; II, insulin index; II/GI, II to GI ratio. Values followed by a different letter in each column were significantly different ($P < 0.05$).

Food & Function Accepted Manuscript

Please do not adjust margins

Food & Function

ARTICLE

Figure captions

Figure 1. Effects of lipid, protein and heating on the different starch fractions of raw materials and cooked ones. Different letters showed the significant differences; Error bars showed standard deviation of at least three replicates.

Figure 2. Mean (± SD) plasma glucose (A) and insulin (B) after ingestion of pure foxtail millet products or standard glucose solution. Error bars showed standard deviation among ten subjects.

Food & Function Accepted Manuscript

Please do not adjust margins

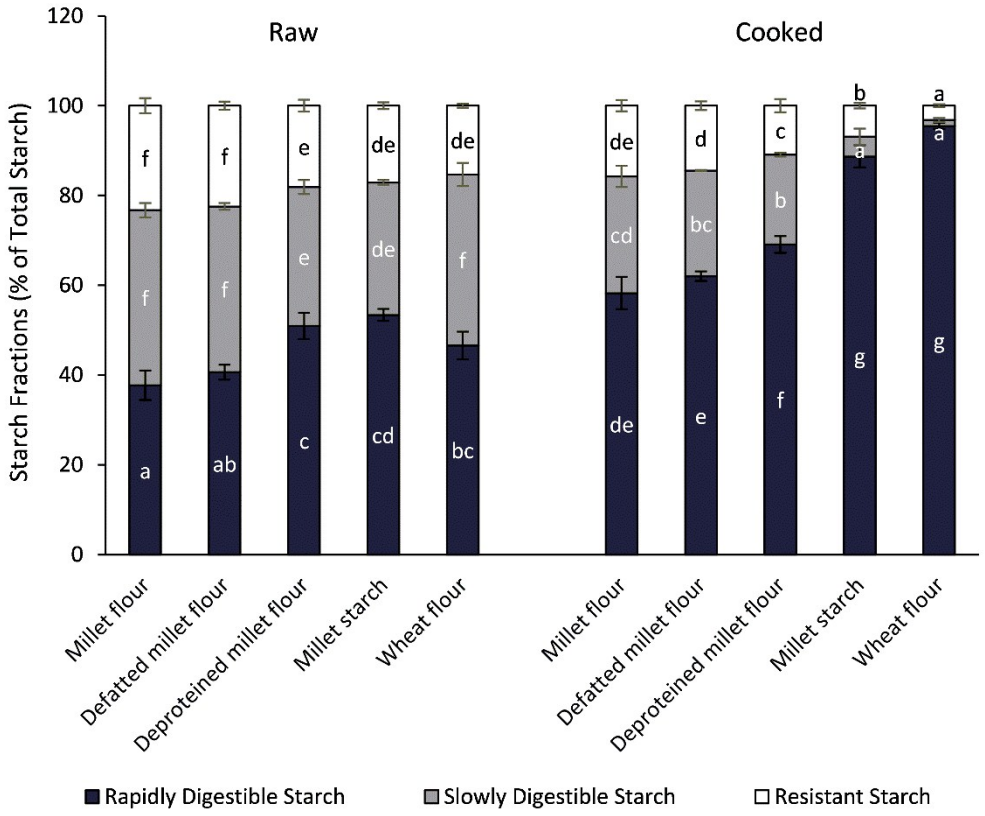


Figure 1. Effects of lipid, protein and heating on the different starch fractions of raw materials and cooked ones. Different letters showed the significant differences; Error bars showed standard deviation of at least three replicates.

Food & Function Accepted Manuscript

Please do not adjust margins

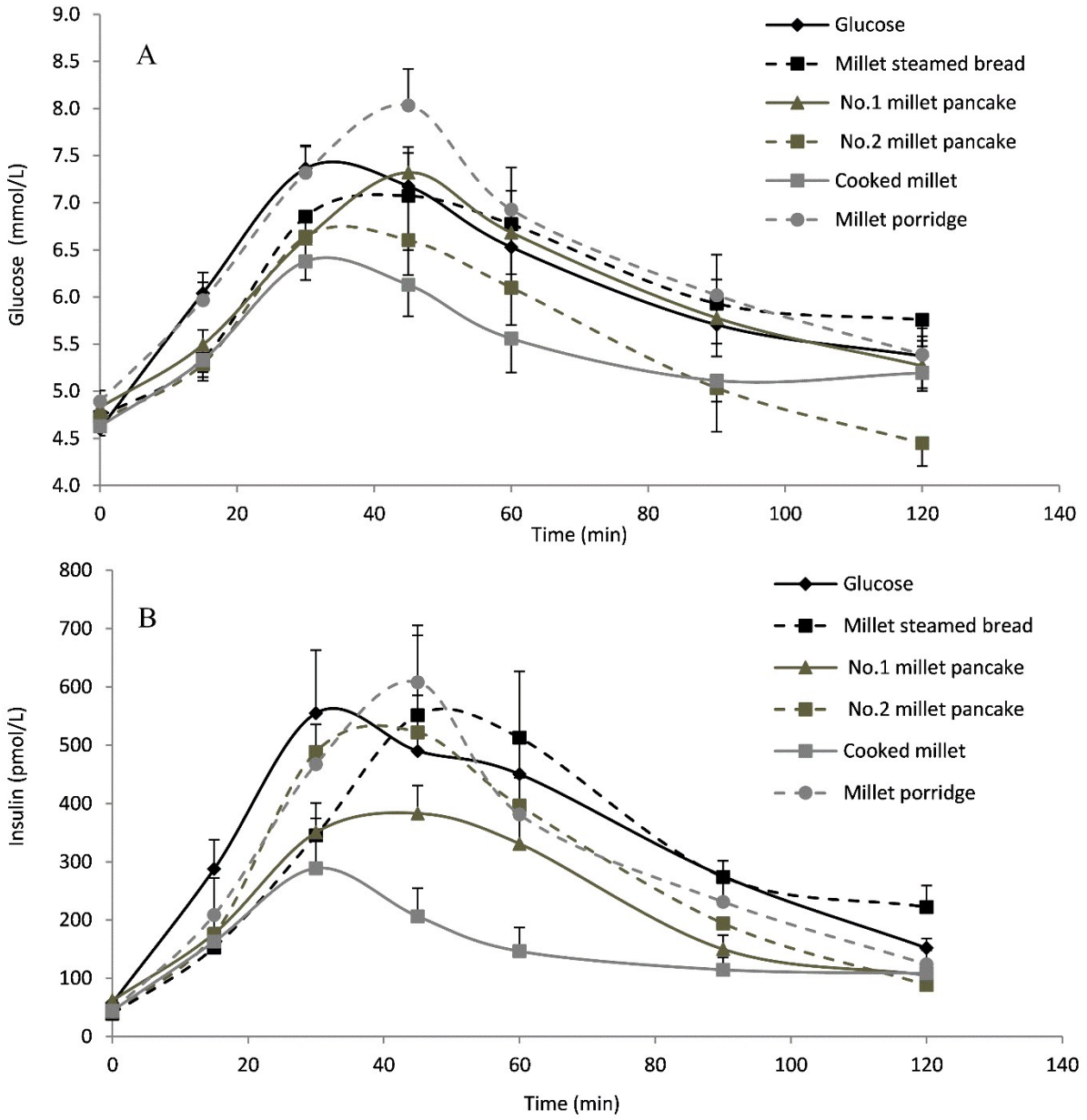
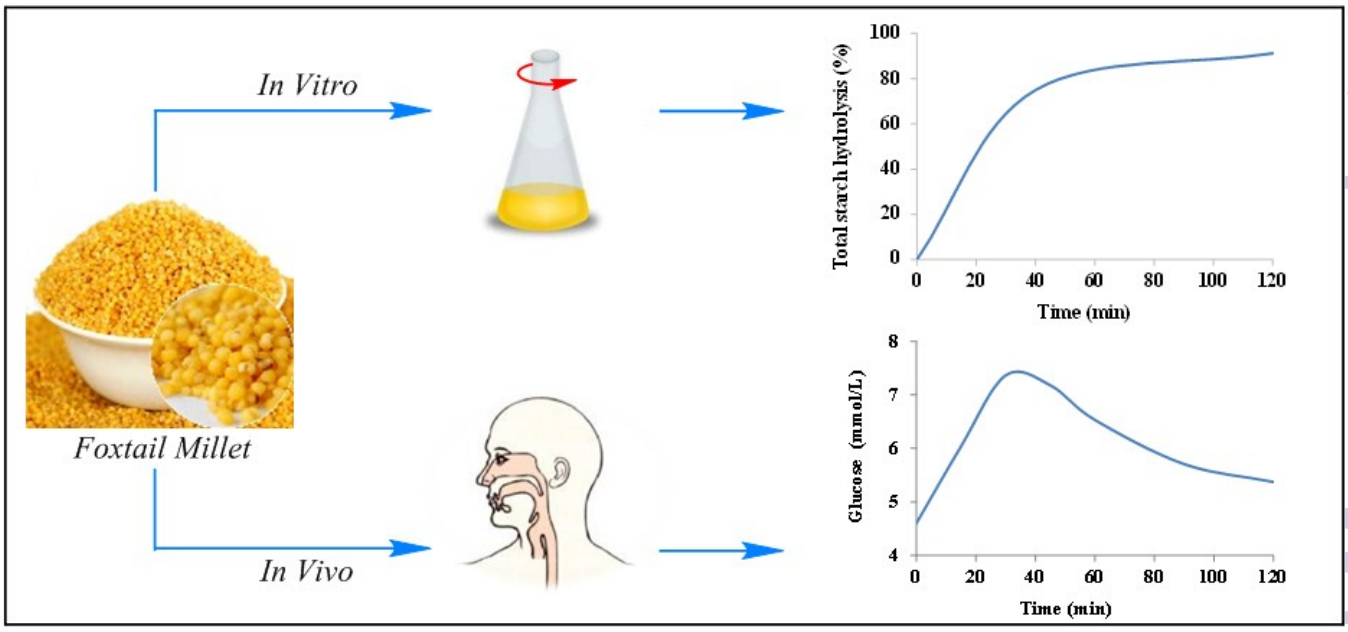


Figure 2. Mean (± SD) plasma glucose (A) and insulin (B) after ingestion of pure foxtail millet products or standard glucose solution. Error bars showed standard deviation among ten subjects.

Food & Function Accepted Manuscript

This journal is © The Royal Society of Chemistry 20xx

Please do not adjust margins



Food & Function Accepted Manuscript

Please do not adjust margins