Understanding the digestibility and nutritional functions of rice starch subjected to heat-moisture treatment

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Abstract: In this study, rice starch with well-controlled digestion resistibility achieved by heat-moisture treatment (HMT) was chosen as a supplementary diet for high-fat-diet-fed mice. Then, the nutritional functions of HMT-modified rice starch were evaluated by the physiological and biochemical indices, proliferation and distribution of intestinal microflora, and functional diversity by putative metagenomes analysis. Compared with the native-rice-starch mice (DM) group, the blood glucose, serum lipid, oxidative stress, and liver function metabolic levels/indices of the HMT-rice-starch mice (HMT-DM) group were worse due to the declined level of slowly digestible starch (SDS) in HMT-modified rice starch. Meanwhile, the species diversity index was observed to be higher in the DM group and *Bifidobacteria* was identified as a type of bacteria related to the relatively higher content of RS in HMT-modified rice starch. Overall, our results provide important information for the rational design of rice starch-based health-promoting foods with nutritional functions.

Keywords: Rice starch; heat-moisture treatment; digestion; nutritional functions; intestinal microflora

Nomenclature

SDS	slowly digestible starch
HMT	heat-moisture treatment
RDS	rapidly-digestible starch
SDS	slowly-digestible starch
RS	resistant starch
TG	triglyceride
TCH	total cholesterol
HDL-c	high-density lipoprotein cholesterol
LDL-c	low-density lipoprotein cholesterol
MDA	Malondialdehyde
SOD	Superoxide dismutase
GSH-PX	Glutathione peroxidase
T-AOC	Total antioxidant capacity
ALT	alanine aminotransferase
ALP	alkaline phosphatase
AST	aspartate aminotransferase
OTUs	Operational Taxonomic Units
SEM	standard error of the mean

1. Introduction

With the improvement of living standard and the increased diversity of diet and disease spectra, foods with nutritional functions such as physiological accommodation and disease prevention have attracted increasing attention (Barratt, Lebrilla, Shapiro, & Gordon, 2017; Caballero, 2013; Link & Reue, 2017). Therefore, designing personalized healthy foods with particular nutritional functions has been one of the hotspots in food science. Rice, one of the most widely consumed staple food, is mainly composed of starch. Modulating the digestibility and nutritional functions of rice starch have a direct impact on human health.

For positive physiological effects and nutritional benefits, starch, depending on the rate and extent of digestion, can be classified into three categories, namely rapidly-digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Dhital, Warren, Butterworth, Ellis, & Gidley, 2017; Englyst, Kingman, & Cummings, 1992). Previous research has shown that RDS, after rapidly digested in the digestive tract, releases glucose, which increases the level of serum glucose, promotes insulin secretion, stimulates the liver and lipocytes to create fat, and increases the serum lipid level in our body (Hung, Chau, & Phi, 2016; Lee et al., 2014). Meanwhile, SDS and RS can retard or restrain the release of glucose, lower the glycemic index, improve the insulin sensitivity, promote the secretion or expression of insulin and adipocyte-secreted factors, and prevent the fat from deposition (Dhital, Bhattarai, Gorham, & Gidley, 2016; Wei, Sissons, Warren, Gidley, & Gilbert, 2016). Interestingly, RS will produce a series of short-chain fatty acid metabolites at a lower pH. Many health benefits and functional properties of RS have been reported including the prebiotic effect on colon microbes (Shang et al., 2017), the adjustment of lipid metabolism (Nathalie et al., 2016), the

improvement in cholesterol metabolism (Newman et al., 2017), and the reduction in the risk of ulcerative colitis (Bindels et al., 2017) and colon cancer (Si, Strappe, Blanchard, & Zhou, 2016; Si, Zhou, Strappe, & Blanchard, 2016). However, gelatinized rice starch consists of over 95% RDS, which can be easily digested and absorbed, leading to a high glycemic response. This negative physiological effect of rich starch does not correspond to the modern concept of nutrition and health. Therefore, recent research has focused on changing the multi-scale structure of rice starch under different ways of processing or physical modification to regulate its digestion, absorption, and metabolism in the human gastrointestinal tract (GIT) and to improve its nutritional functions.

Different ways of changing the starch multi-scale structure and regulating the starch digestibility have been widely reported (Klein et al., 2013; Puncha-Arnon & Uttapap, 2013; Silva et al., 2017; Tan et al., 2017). As a new physical modification method, heat-moisture treatment (HMT) only involves the use of thermal energy and moisture, which has advantages such as environmental protection, high efficiency, and safety (Wang, Zhang, Chen, & Li, 2016; Zavareze & Dias, 2011). HMT has already played an important role in the green processing of starch (Arns et al., 2015; Hung, Vien, & Phi, 2016; Pratiwi, Faridah, & Lioe, 2017; Silva et al., 2017). However, limited work has been undertaken for evaluating the effect of HMT on the nutritional functions of starch in terms of blood lipid metabolism, blood sugar metabolism, and intestinal microflora. Thus, it is necessary to establish a systemic connection between digestibility and nutritional functions, which would contribute to the development of healthy starch food by HMT.

Therefore, in this study, we investigated the physiological and biochemical indexes and the proliferation and distribution of intestinal microbiota for 10 high-fat-diet-fed mice supplemented

with rice starch (the DM group) and another 10 with HMT-modified rice starch (the HMT-DM group) to understand the alteration of nutritional functions by HMT. To the best of our knowledge, it is the first study comprehensively comparing these detailed nutritional function parameters between native rice starch and HMT-modified rice starch.

2. Material and methods

2.1 sample preparation

Native rice starch was purchased from the South China Agricultural University (Guangzhou, China). The RDS, SDS and RS contents of this starch were 32.6%, 52.4%, 9.0%, respectively, which was analyzed with a modified Englyst procedure according to a previous study in our lab (Wang et al., 2018). The HMT of rice starch was conducted under a moisture content of 25%. The sample was equilibrated at 4 °C for 24 h. Then, they were placed in a 500 mL screwed stainless steel reactor with continuous rotation and heated with oil at 110 °C for 4 h, followed by cooling to room temperature. During HMT, the starch granules were stirred by the paddle when the reactor was continuously rotated. Subsequently, the treated samples were dried at 40 °C and then ground. The RDS, SDS and RS contents of HMT-modified rice starch were measured to be 60.4%, 20.7%, 18.9%, respectively.

2.2 Animals

Twenty healthy male C57BL-6 mice (non-obese) of 20±5 g weight were purchased from the animal house, the Academy of Military Medical Sciences (China). These mice were fed in a clean-grade facility in the Laboratory Animal Center at Tianjin University of Science and Technology.

After one week's adaptive feeding with a basal diet, no difference in the body weight was observed. These mice were divided into two groups randomly. One group of mice were fed a high-fat diet supplemented with rice starch (the DM group) and the other group supplemented with HMT-modified rice starch (the HMT-DM group). For both groups, the intervention was implemented for 8 weeks. Their body weights and food intakes were monitored weekly. Feces were collected on the day of necropsy.

All animal procedures were approved by the Ethical Committee for the Experimental Use of Animals in the Center for Drug Safety Evaluation, Tianjin University of Science & Technology (Approval No: 13/051/MIS).

2.3 **Experimental diets**

The experimental high-fat diet was prepared by Research Diets (Choi, Gwon, Ahn, Jung, & Ha, 2013), which consisted of 54.5% carbohydrate source, 10% lard oil, 20% casein, 5% corn oil, 0.5% cholesterol, 5% cellulose, 3.5% mineral mix (based on AIN76), 1% vitamins (based on AIN76), 0.3% methionine, and 0.2% choline bitartrate. The two groups of mice were fed with the experimental high-fat diet where carbohydrate source was replaced by rice starch and HMT-modified rice starch, respectively.

2.4 **Blood and tissue analysis**

At the end of the intervention period, the mice were fasted overnight, before the blood glucose was recorded. Blood samples were taken from the arteria femoralis for the determination of physiological and biochemical indexes. The liver tissue, epididymal white adipose tissue, and perirenal white

adipose tissue were quickly removed after sacrifice and then weighed. The liver tissue was immediately stored at −80 °C for further pathological slides.

2.4.1 Analysis of blood lipids composition

Blood lipid indexes evaluated in this study included triglyceride (TG), total cholesterol (TCH), high-density lipoprotein cholesterol (HDL-c), and low-density lipoprotein cholesterol (LDL-c). The specific measurement procedures and final calculations were performed according to the specification of the TG, TCH, HDL-c, and LDL-c assay kits (Jian Cheng Biotechnology Co., Ltd., Nanjing, China).

2.4.2 Analysis of oxidative stress index

There are four main indexes for characterizing oxidative stress. Malondialdehyde (MDA) is a final product of the lipid oxidation process and was measured according to an MDA assay kit (Jian Cheng Biotechnology Co., Ltd., Nanjing, China). Superoxide dismutase (SOD) was determined using an SOD assay kit (Jian Cheng Biotechnology Co., Ltd., Nanjing, China). Glutathione peroxidase (GSH-PX) was measured using an assay kit (Jian Cheng Biotechnology Co., Ltd., Nanjing, China) and the activity was calculated according to the manufacturer's instructions. The Total antioxidant capacity (T-AOC) was determined using a T-AOC assay kit (Jian Cheng Biotechnology Co., Ltd., Nanjing, China) according to the manufacturer's instructions.

2.4.3 Determination of hepatic lipid metabolism enzymes

The detection of the liver functional metabolism mainly includes three indices reflecting the aspartate transaminase (Nathalie et al.), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) activities. The aspartate aminotransferase (AST) activity was measured by colorimetric analysis using an AST assay kit (Jian Cheng Biotechnology Co., Ltd, Nanjing, China). The ALT activity was measured by colorimetric analysis using an ALT assay kit (Jian Cheng Biotechnology Co., Ltd, Nanjing, China). The ALP activity was measured by standard oxidation using potassium ferricyanide.

2.4.4 Liver histology and analysis

The liver tissues were placed in 10% neutral formalin liquid for 12 h, which were then dehydrated using 30%, 50%, 70%, 80%, 90%, 95% and 100% ethanol solutions, respectively, and washed in xylol. Then, the liver tissues were embedded in paraffin (BMJ-III embedding machine, Jiangsu, China). Finally, they were stained with hematoxylin and eosin (H & E) and observed by light microscopy at 200× magnification.

2.5 Microbial community analysis

2.5.1 Feces samples collection, DNA extraction, and 16S rRNA gene sequencing

Feces samples were collected in a sterile container and immediately stored at −80 °C until further processing. Total genome DNA from samples was extracted using the hexadecyltrimethylammonium bromide/sodium dodecyl sulfate (CTAB/SDS) method (Caporaso et al., 2010). The DNA

concentration and purity were monitored on 1% agarose gels. The 16S rRNA genes of distinct regions in V4 were amplified using the specific primer of 515F-806R. All polymerase chain reaction (PCRs) were carried out with a Phusion® High-Fidelity PCR Master Mix (New England Biolabs). The same volumes of 1× loading buffer (containing SYBR Green I) were mixed with PCR products and electrophoresis was operated on 2% agarose gels. Sequencing libraries were generated using a TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA) following manufacturer's recommendations, and index codes were added. Finally, the library was sequenced on an IlluminaHiSeq2500 platform and 250 bp paired-end reads were generated.

2.5.2 Intestinal microbiota analysis

After the removal of the low-quality sequences, pyrosequencing errors, and chimera, all the sequencing reads were denoised. Sequences of >97% similarity levels were assigned to the same Operational Taxonomic Units (OTUs) (Edgar, 2013). Representative sequences for each OTU were screened for further annotation. The rare fraction curves and alpha diversity indices (Chao1, ACE, Shannon, and Simpson) were performed using MOTHUR software, and the beta diversity (among samples) was analyzed using principal component analysis (PCA). The function of intestinal microbiota was performed by online phylogenetic investigation of communities by reconstruction of an unobserved states program (PICRUSt, http://picrust.github.io/picrust/).

2.6 Statistical analysis

All analyses were conducted in triplicate and statistical analyses were performed using the Statistical Package for Social Science (SPSS) software version 22. Body weight and food intake

were analyzed using General Linear Model repeated measures (Mixed Design ANOVA). *P* interaction indicates the effect of time and group interactions. Once a significant difference ($P \leq 0.05$) was detected, post hoc multi-comparisons were performed by LSD adjustment. Other physiological and biochemical indexes (blood glucose and insulin) were analyzed by one-way analysis of variance followed by Tukey's multiple comparison analysis. Different lowercase letters above the same column indicate a significant difference ($P \leq 0.05$). Results were expressed as mean ± standard error of the mean (SEM).

3. Results and discussions

3.1 Body weight, and liver and adipose tissue weights

Fig. 1 shows the changes in body weight of the two groups of mice after the 8-week intervention. The initial body weights between the two groups were not significantly different ($P > 0.05$), showing the rationality of the grouping method. From the start to Week 4, there were apparent decreasing trends of the body weight and food intake for both of the groups. Afterwards, both the body weight and food intake gradually increased. At the end of Week 8, the body weights of mice in these two groups were 30% higher than their initial values, and General Linear Model (GLM) analysis showed no significant difference (week × group interaction) between the two groups ($P > 0.05$).

After the 8-week intervention, the perirenal fat, epididymal fat, and total fat-to-body ratio of the HMT-DM group mice were significantly higher than those of the DM group mice (**Table 1**). These

results indicate that native rice starch had better performance in controlling the fat tissue deposit in contrast to HMT-modified rice starch.

3.2 **Blood glucose, insulin and lipid levels**

Table 2 shows the blood glucose, insulin, and blood lipid levels of the two groups of mice. Previous research has shown that the blood glucose level of C57BL-6 mice was normally 6–10 mmol/L (Rodríguez, Limón-Pacheco, Del Razo, & Giordano, 2016), which was slightly lower than those of two experimental groups after the 8-week intervention. This observation indicated that both native rice starch and HMT-modified rice starch could stabilize the blood glucose level and reduce the speed of change into a hyperglycemia condition. In addition, the blood glucose level of the HMT-DM group was significantly higher than that of the DM group ($P < 0.05$), which might be related to the increased RDS content and greatly decreased SDS content after HMT. The result confirmed that the SDS content has a strong impact on the regulation of the blood glucose level as also shown in recent studies (Kittisuban, Lee, Suphantharika, & Hamaker, 2014; Wolever et al., 2016).

After the intervention, the serum insulin levels of these two groups were all within the normal range (Seghers, Nakazaki, Aguilar, & Bryan, 2000). Nonetheless, compared with the DM group, the HMT-DM group displayed a significantly higher serum insulin level ($P < 0.05$), indicating a higher insulin resistance of the HMT-DM group than that of the DM group. Thus, the HMT-modified rice starch intervention reduced the biological effect of insulin as well as the sensitivity of the insulin receptor tissue to insulin.

According to a previous study (Roza, Possignolo, Palanch, & Gontijo, 2016), the degree of dyslipidemia and the levels of TG, TC and LDL-c were all positively proportional to obesity, while

231 the HDL-c level was negatively related to obesity. It has been proved that the abnormal deposition of
232 TG in the liver and skeletal muscles can impair the activity of oxidase and affect the metabolism of
233 glucose and lipids (Zhang, 2016). In addition, it is widely believed that the abnormal changes in
234 individual blood lipid levels, such as increased TC, TG and LDL-c levels and a reduced HDL-c level,
235 could be the causes of coronary heart disease (Sugawara et al., 2000). In other words, the blood
236 lipids have a strong relationship with health. **Table 2** summarizes the blood lipid levels of the two
237 groups at the end of the intervention period. It can be seen that the TC and TG levels of blood lipids
238 in the HMT-DM group were significantly higher than those of the DM group ($P < 0.05$), while the
239 HDL-c level was significantly lower ($P < 0.05$). These results suggest that after the high-fat-diet
240 intervention for 8 weeks, the blood lipid indices of mice in the DM group were better than those of
241 HMT-DM group. In other words, the ability of rice starch in regulating the blood lipid levels was
242 actually reduced by HMT.

243 ### 3.3 **Oxidative stress indices**

244 Oxidative stress causes cytotoxicity and the accumulation of reactive oxygen species in the
245 body (Reeves, Nielsen, & Fahey, 1993). In this study, the changes in MDA, SOD, GSH-PX and T-
246 AOC levels in the serum of mice were examined to understand the influence of native and HMT-
247 modified rice starches on these oxidative stress indices. After a high-fat diet intake, a large amount of
248 calorie led to exuberant energy metabolism and hyperglycemia status in mice, which then intensified
249 glucose oxidation to produce excessive oxidative products (Cristani et al., 2016). Meanwhile, the
250 stronger oxidative ability of excessive oxidative products than the antioxidant capacity could lead to
251 oxidative stress in the body. The reason for the reduced amounts of antioxidant enzymes could be the

252 rapid consumption and exhaustion of their storage in the body when fighting free radicals generated
253 during development of obesity (Sathiavelu, Senapathy, Devaraj, & Namasivayam, 2009). The results
254 (**Table 3**) show a statistically significant increase in serum levels of MDA and GSH-PX, as well as a
255 decrease in T-AOC level for the HMT-DM group compared with for the DM group (P <0.05).
256 Overall, the serum oxidative stress status of mice in the DM group was more effectively improved
257 than that of mice in the HMT-DM group. Thus, it could be suggested that native rice starch,
258 compared with HMT-modified rice starch, could better suppress oxidant stress induced by the high-
259 fat diet.

260 ### 3.4 **hepatic lipid metabolism enzymes**

261 The detection of hepatic metabolism enzymes mainly includes ALT, AST, and ALP. In the
262 present study, the activities of AST, ALT, and ALP are increased in the serum, which can be
263 considered an indication of liver damage. Previous studies have shown that cell membrane damage
264 leads to a more prominent increase in ALT and AST activities (Borlak, Chougule, & Singh, 2014).
265 The data in **Table 3** also show that the ALT and AST levels of mice in the HMT-DM group were
266 significantly higher than those in the DM group (P < 0.05). Thus, the increased levels of these
267 enzymes indicated that HMT-modified rice starch may have a significant impact on cellular
268 homeostasis. Overall, these results suggest that compared to the intervention with the HMT-modified
269 rice starch, native rice starch could alleviate liver dysfunction to a certain degree, and could prevent
270 or delay the abnormal liver function caused by fat accumulation.

271 **Fig. 2** shows that the liver tissue of mice in the DM group had a large amount of fatty
272 degeneration, with different sizes of lipid droplet vacuoles (marked by black circles) in the cytoplasm

and some degree of perivascular infiltration by inflammatory cells. While the liver tissue of the HMT-DM group mice had visible steatosis as well, it contained fewer lipid vacuoles, higher vascular permeability, and more perivascular infiltration than that of DM group did. In other words, the resistance to abnormal liver function caused by the high-fat diet was reduced by the intervention with HMT-modified rice starch.

3.5 **Intestinal microflora**

The gut is a complex, active, relatively balanced system, of which the type and flora amounts can be changed when the body acquires different kinds of flora from the environment and food (Clemente, Ursell, Parfrey, & Knight, 2012). Recent research has revealed the function of intestinal microflora for the normal homeostasis of the human body (Hartstra, Nieuwdorp, & Herrema, 2016). **Fig. 3A** shows the gut microbiota composition in mice after the 8-week intervention for the two groups. The observed species indices in the DM group was significantly higher than those in the HMT-DM group ($P < 0.05$), which indicates a higher intestinal flora abundance of the DM group. This result confirmed that when RS reached the colon and degraded by microbes, the degradation products would change the distribution structure of intestinal flora and reduce its species abundance. In addition, the PCA analysis diagram based on OTU abundance showed two relatively separated macroscopic distribution profiles, accounting for the apparent difference in the microbial structure between the DM group and the HMT-DM group. To further analyze the difference in microbial structure between the two groups, a weighted UniFrac distance matrix was constructed (shown in **Fig. 3B**). Interestingly, the difference within the HMT-DM group was larger than that within the DM group based on the level of a single sample of OTUs.

294 Then, we analyzed the statistical results of species percentage among different species
295 classification levels. At the phylum level (shown in **Fig. 3C**), bacteria between the two groups were
296 mainly composed of *Bacteroidete*, *Firmicutes*, and *Proteobacteria*. These three bacteria took up
297 about 45.8%, 31.6% and 17.7% in the DM group mice whereas those in the HMT-DM group mice
298 were 45.2%, 24.3.0%, and 24.4%, respectively. Thus, there were significant differences in the
299 percentage of *Firmicutes* and *Proteobacteria* between the two groups (P <0.05).
300 At the family level, the microbial structure and relative abundance in the two groups of mice were
301 also shown in **Fig. 3C**. The proportions of *Ruminococcaceae*, *Lachnospiraceae*,
302 *Porphyromonadaceae*, and other bacteria in the DM group were slightly higher than that in the
303 HMT-DM group. The ratios were 7.1% *vs.* 4.2%, 7.6% *vs.* 4.1%, and 3.6% *vs*. 1.9% (DM *vs.* HMT-
304 DM), respectively (P <0.05). At the same time, the proportions of some species of the intestinal
305 microflora in the HMT-DM group, namely *Helicobacteraceae*, *S24-7*, *Erysipelotrichacea*,
306 *Desulfovibrionaceae*, and *Bifidobacteriaceae*, were significantly higher than that in the DM group,
307 and the ratios were 17.5% *vs.* 6.6%, 23.0 *vs.* 18.6%, 10.6% *vs.* 6.4%, 4.5% *vs.* 1.3% (HMT-DM vs.
308 DM), respectively (P <0.05). Studies have shown that the digestive tract adenoma or cancer of the
309 organism are correlated positively with *Ruminococcaceae*, *Porphyromonadaceae*, and
310 *Lachnospiraceae (Frank et al., 2007; Meehan & Beiko, 2014)*, and negatively with *Bacteroides*,
311 *Bifidobacteriaceae* and *Desulfovibrionaceae* (Panasevich et al.). Therefore, it could be speculated
312 that when RS entered the colon and degraded by microbes, the degradation products could
313 effectively inhibit the growth of some species of intestinal bacteria such as *Ruminococcaceae*,
314 *Porphyromonadaceae* and *Lachnospiraceae*, but meanwhile stimulate the growth of *Bacteroides*,

315 *Bifidobacteriaceae* and *Desulfovibrionaceae*, which could suppress the occurrence of digestive tract
316 adenoma and cancer.
317 Metagenomics analysis using the linear discriminant analysis effect size (LEfSe) method was
318 performed to compare the microbial community composition and its abundance diversity in feces
319 between the two groups of mice from the phylum, class, order, family and genus levels, and to
320 further select the dominant flora of microbial communities in the two groups. It can be seen in **Fig. 4**
321 that there were large differences in each level of microbial communities between the two groups.
322 According to the LDA score, the dominant bacteria in the HMT-DM group were *Bifidobacterium*,
323 while *S24-7*, *β-proteobacteria*, and *Proteobacteria* occupied important places in the DM group.
324 Some previous opinions considered that *bifidobacteria* were one of the probiotics associated with the
325 glucose and lipid metabolism (Martorell et al., 2016; Patterson et al., 2017). When the disorder of
326 glucose and lipid metabolism occurred, there was a decrease in the *bifidobacteria* content in the gut.
327 In contrast, a higher amount of *bifidobacteria* contributed to normalizing the blood glucose and
328 blood lipid levels. However, here, the content of *bifidobacteria* in the HMT-DM group mice was
329 higher than that in the DM group, though the indices on blood glucose and blood lipids in the HMT-
330 DM group mice were still worse than those in the DM group (see **Table 1** and **Table 2**). These
331 differences were mainly due to the high sugar environment produced by the high-fat diet, which led
332 to lower activity of functional enzymes in the gut. Therefore, SDS showed more powerful effects on
333 regulating blood glucose and lipids.

Besides, *Parabacteroides* and *S24-7* were pathogenic bacteria, which were closely related to the incidence of cancer in the digestive tract. Our results showed that HMT-modified rice starch could effectively inhibit the pathogenic bacteria, and reduce the occurrence of cancer in the digestive tract.

The results of animal experiments in our study indicate that physiological and biochemical indices were mainly depended on the content of enzyme-resistant components (SDS + RS) in rice starch, whilst the intestinal microflora was determined by the RS content.

We also assessed the functional diversity of the different putative metagenomes using PICRUSt software (Langille et al., 2013), which allows the prediction of metabolic pathways from the 16S rRNA reads. Most of the genes were detected to annotate in the pathways of environmental information processing, genetic information processing, and metabolism. Moreover, the pathways displayed a difference in the mean proportion between the DM and HMT-DM groups (**Fig. 5**). Some pathways including carbohydrate metabolism, lipid metabolism, and membrane transport were over-represented in the DM group, whereas the metabolisms of cofactors, vitamins and amino acids and the cell motility were over-represented in the HMT-DM group. These results indicate that rice starch associated with HMT may also influence the functional diversity, especially predicted by putative metagenomes.

4. Conclusions

This research is focused on the nutritional function of rice starch after HMT, and the relationship between digestibility and nutritional function was established. The results indicate that the physiological and biochemical indices of the HMT-DM group were slightly worse than those of the DM group due to the relatively lower content of SDS in HMT-modified rice starch. *Bifidobacteria*

were identified as a type of bacteria related to HMT-modified rice starch, which might be ascribed to the relatively higher content of RS in HMT-modified rice starch, although the specific functions of this type of bacteria require further studies. Moreover, some pathways influenced by these two starches were annotated to reveal the functional metabolisms in the body. Thus, this work is of great significance in modulating the nutritional functions of rice starch and is instrumental to the development of starch-based healthy food.

Potential conflict of interest statement

The authors declare no competing financial interest.

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Figure captions

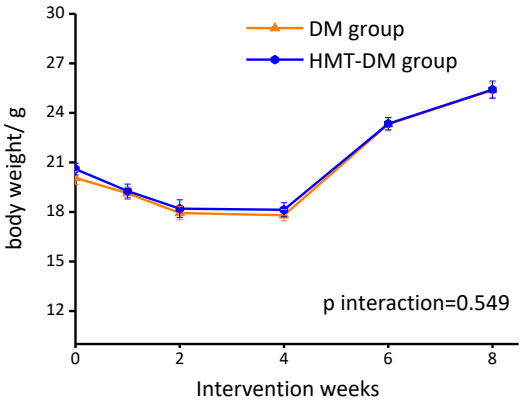
Figure 1. Changes in body weight and food intake of mice during the 8-week intervention for the two groups. Statistical analysis was performed by General Linear Model repeated measures (Mixed Design ANOVA). P interaction indicates the effect of time and group interactions.

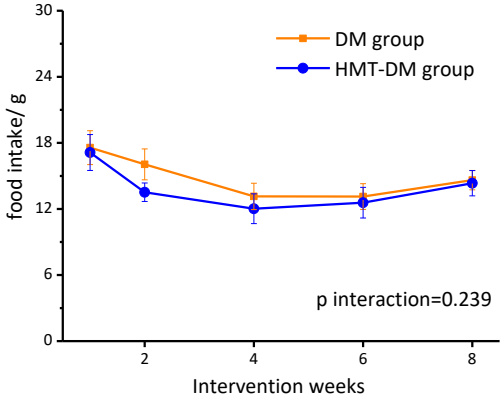
Figure 2. Light microscope images of liver tissue of mice after the 8-week intervention for the two groups (A: DM group; B: HMT-DM group)

Figure 3. Gut microbiota composition in mice after the 8-week intervention for the two groups. A: Alpha diversity index; B: β diversity of intestinal microflora (left: PCA analysis; right: analysis of weighted UniFrac distance matrix) (adjusted *P* value < 0.05); C: The relative abundance of bacterial community at the taxa level (left: Phylum; right: Family) for the two groups (C1: DM group; C2 HMT-DM group)

Figure 4. Significant analysis of intestinal microflora in mice for the two groups (C1: DM group; C2: HMT-DM group)

Figure 5. Function in KEGG module prediction using 16S data with PICRUSt. (A: KEGG genes annotation; B: different notability functions between the two groups; C1: DM group; C2: HMT-DM group).


DM group
HMT-DM group
body weight/ g
Intervention weeks
p interaction=0.549


DM group
HMT-DM group
food intake/ g
Intervention weeks
p interaction=0.239

527

Figure 1

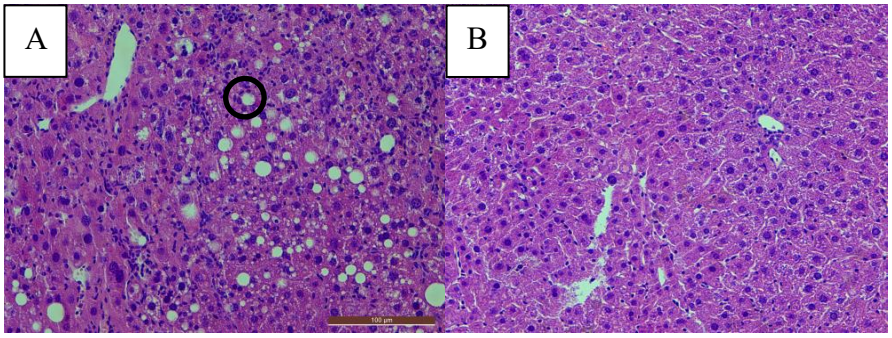
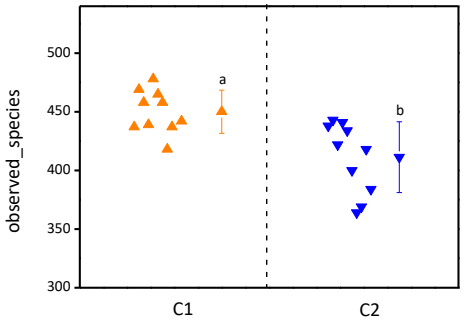
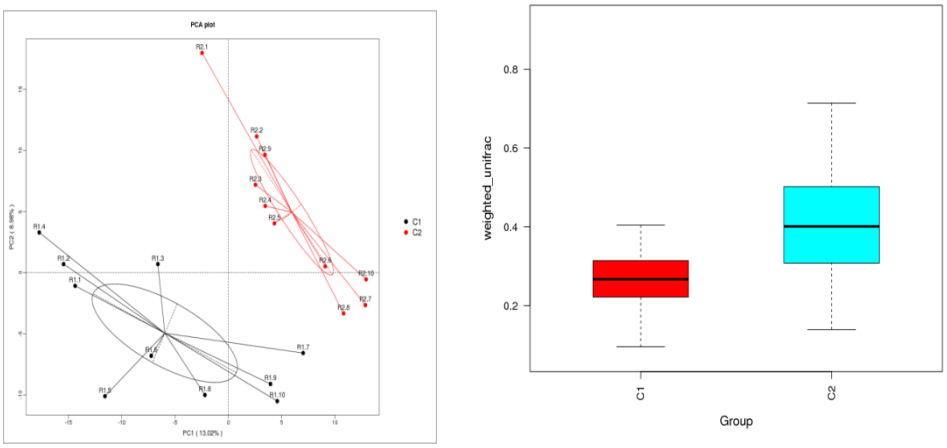


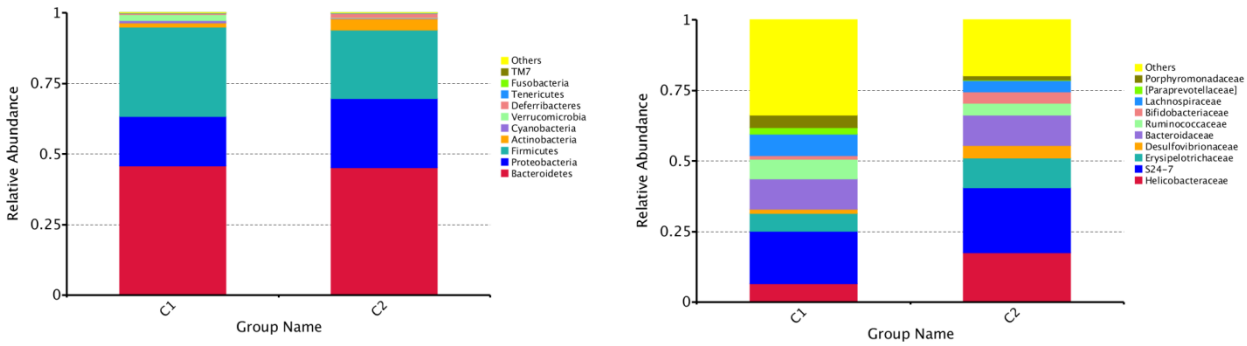
Figure 2


observed_species
500
450
400
350
300
a
b
C1
C2

(A)

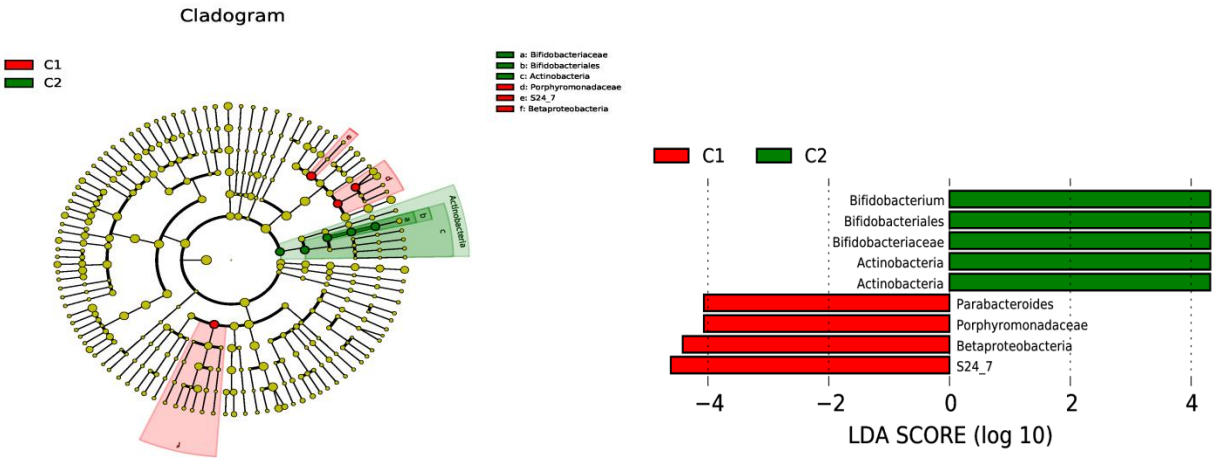

PCA plot
C1
C2
weighted_unifrac
0.8
0.6
0.4
0.2
C1
C2
Group

(B)


Relative Abundance
1
0.75
0.5
0.25
0
C1
C2
Group Name
Others
TM7
Fusobacteria
Tenericutes
Deferribacteres
Verrucomicrobia
Cyanobacteria
Actinobacteria
Firmicutes
Proteobacteria
Bacteroidetes
Relative Abundance
Group Name
Others
Porphyromonadaceae
[Paraprevotellaceae]
Lachnospiraceae
Bifidobacteriaceae
Ruminococcaceae
Bacteroidaceae
Desulfovibrionaceae
Erysipelotrichaceae
S24-7
Helicobacteraceae

(C)

Figure 3


Cladogram
C1
C2
a: Bifidobacteriaceae
b: Bifidobacteriales
c: Actinobacteria
d: Porphyromonadaceae
e: S24_7
f: Betaproteobacteria
Actinobacteria
C1
C2
Bifidobacterium
Bifidobacteriales
Bifidobacteriaceae
Actinobacteria
Actinobacteria
Parabacteroides
Porphyromonadaceae
Betaproteobacteria
S24_7
−4
−2
0
2
4
LDA SCORE (log 10)

(A) (B)

Figure 4

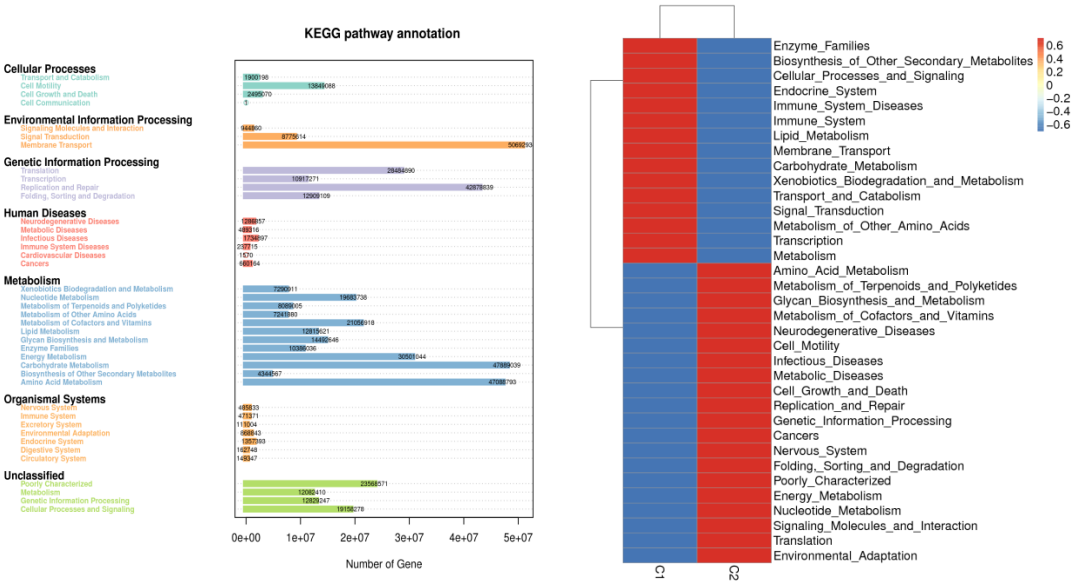

KEGG pathway annotation
Cellular Processes
Transport and Catabolism
Cell Motility
Cell Growth and Death
Cell Communication
Environmental Information Processing
Signaling Molecules and Interaction
Signal Transduction
Membrane Transport
Genetic Information Processing
Translation
Transcription
Replication and Repair
Folding, Sorting and Degradation
Human Diseases
Neurodegenerative Diseases
Metabolic Diseases
Infectious Diseases
Immune System Diseases
Cardiovascular Diseases
Cancers
Metabolism
Xenobiotics Biodegradation and Metabolism
Nucleotide Metabolism
Metabolism of Terpenoids and Polyketides
Metabolism of Other Amino Acids
Metabolism of Cofactors and Vitamins
Lipid Metabolism
Glycan Biosynthesis and Metabolism
Enzyme Families
Energy Metabolism
Carbohydrate Metabolism
Biosynthesis of Other Secondary Metabolites
Amino Acid Metabolism
Organismal Systems
Nervous System
Immune System
Excretory System
Environmental Adaptation
Endocrine System
Digestive System
Circulatory System
Unclassified
Poorly Characterized
Metabolism
Genetic Information Processing
Cellular Processes and Signaling
0e+00
1e+07
2e+07
3e+07
4e+07
5e+07
Number of Gene
Enzyme_Families
Biosynthesis_of_Other_Secondary_Metabolites
Cellular_Processes_and_Signaling
Endocrine_System
Immune_System_Diseases
Immune_System
Lipid_Metabolism
Membrane_Transport
Carbohydrate_Metabolism
Xenobiotics_Biodegradation_and_Metabolism
Transport_and_Catabolism
Signal_Transduction
Metabolism_of_Other_Amino_Acids
Transcription
Metabolism
Amino_Acid_Metabolism
Metabolism_of_Terpenoids_and_Polyketides
Glycan_Biosynthesis_and_Metabolism
Metabolism_of_Cofactors_and_Vitamins
Neurodegenerative_Diseases
Cell_Motility
Infectious_Diseases
Metabolic_Diseases
Cell_Growth_and_Death
Replication_and_Repair
Genetic_Information_Processing
Cancers
Nervous_System
Folding,_Sorting_and_Degradation
Poorly_Characterized
Energy_Metabolism
Nucleotide_Metabolism
Signaling_Molecules_and_Interaction
Translation
Environmental_Adaptation
0.6
0.4
0.2
0
−0.2
−0.4
−0.6
C1
C2

Figure 5

Table 1 Changes in perirenal fat, epididymal fat, fat-to-body ratio, and organ coefficient of mice after the 8-week intervention for the two groups (mean ± SEM).

Group	Organ coefficient	Perirenal fat (g)	Epididymal fat (g)	Fat ratio (100%)
DM	0.054±0.0063[a]	0.094±0.0074 [a]	0.418±0.0139[a]	2.084±0.10[a]
HMT-DM	0.058±0.0065[a]	0.120±0.0102 [b]	0.464±0.0105[b]	2.119±0.13[b]

Different lowercase letters above the same column indicate a significant difference ($P \leq 0.05$).

Table 2 Changes in blood glucose, insulin levels, and serum lipid of mice after the 8-week intervention for two groups (mean ± SEM).

Groups	Blood glucose (mmol/L)	Insulin (mU/L)	TC (nmol/L)	TG (nmol/L)	HDL-c (μmol/L)	LDL-c (μmol/L)
DM	11.12±0.28[b]	0.115±0.002[b]	1.922±0.059[b]	12.629±0.789[b]	104.676±5.310[a]	88.551±2.384[a]
HMT-DM	12.62±0.37[a]	0.123±0.002[a]	1.990±0.065[a]	15.644±0.200[a]	97.523±1.753[b]	88.855±1.690[a]

Different lowercase letters above the same column indicate a significant difference ($P \leq 0.05$).

Table 3 Changes in oxidative stress and liver function metabolic levels of mice after the 8-week intervention for two groups (mean ± SEM).

Groups	MDA (nmol/mL)	SOD (U/mL)	GSH-PX (U/mL)	T-AOC (U/mL)	ALT (U/L)	AST (U/L)	ALP (IU/L)
DM	7.26±0.42^{b}	139.89±9.35^{a}	600.00±46.82^{b}	10.38±0.68^{a}	12.47±1.98^{b}	124.07±11.78^{b}	0.11±0.01^{a}
HMT-DM	8.41±0.99^{a}	141.50±5.68^{a}	715.86±35.47^{a}	9.52±0.23^{b}	15.15±0.88^{a}	137.13±11.15^{a}	0.12±0.01^{a}

Different lowercase letters above the same column indicate a significant difference ($P \leq 0.05$).