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Potential effects of dietary Maillard reaction products derived from 1 to 3 kDa soybean peptides on the aging ICR mice

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ABSTRACT

Effects of Maillard reaction products derived from 1 to 3 kDa soybean peptides (MRPF3) on aging ICR mice were investigated. Seven animal groups were established for 5 weeks, including one normal group and six D-galactose (1000 mg kg^{-1}/day) treated groups. Aging control was D-galactose + saline solution, and positive controls were D-galactose + ascorbic acid (Vc) (400 mg kg^{-1}/day) and oligofructose (400 mg kg^{-1}/day), respectively, while the test groups are D-galactose + high (800 mg kg^{-1}/day), medium (400 mg kg^{-1}/day) and low (200 mg kg^{-1}/day) doses of MRPF3. Compared with the aging controls, food intake, body weights and organ indexes returned to normal after feeding with MRPF3, and the color of feces as well as the fluorescence intensity of urine increased. The content of malondialdehyde (MDA) in the liver significantly decreased with the intake of MRPF3, and the activities of SOD and GSH-Px and the total antioxidant capacity of serum significantly increased. The abundance ratio of *Bacteroidetes and Firmicutes* significantly decreased in MRPF3 groups, and the abundance of *Lactobacillus* significantly increased, while potentially pathogenic bacteria such as *Porphyromonadaceae* significantly decreased. Our results showed that MRPF3 might offer a potent retardation potential for aging.

1. Introduction

Currently, fried, roasted and baked foods are the main components for consumers in fast-food diets, and the attractive color and flavors are mainly produced by the Maillard reaction (MR) during the food processing (Jaeger et al., 2010). Ogasawara et al. (2006) found that flavors, including umami, "mouthfulness", and continuity in the umami solutions were significantly enhanced via using Maillard reaction products (MRPs) derived from 1000 to 5000 Da soybean peptides. Some unique aromas, such as roasted, meaty and caramel flavors, have been confirmed to be developed by MRPs (Domínguez et al., 2014). The presence of MRPs in flavor enhancers have made foods more attractive to consumers, and this has stimulated the recent increase in MRP consumption (Wu et al., 2018).

In our previous study, MRPs derived from 1 to 3 kDa soybean peptides (MRPF3) were prepared to have better sensory characteristics with strong meaty and umami flavors and less bitter taste. Furthermore, much better antioxidant activity was obtained in MRPF3, as 79.76% of DPPH (2,2-diphenyl-1-picryl-hydrazylhydrate) radicals could be eliminated at the low MRP concentration of 0.08 mg/ml (Yu et al., 2018). Similarly, good DPPH radical-scavenging activity and reducing power *in vitro* of MRPs were confirmed in the earlier study, which were prepared by heating the mixture of soybean peptides and D-xylose (Na et al., 2014). In detail, melanoidins are formed during MR, and exert efficient *in vitro* antioxidant activities, especially for the generation of low molecular weight MRPs because of the polymerization and dehydration reactions (Rufián-Henares and Morales, 2007). Melanoidins have been confirmed to be inhibiting the lipid peroxidation induced by adriamycin in hepatocytes (Valls-Bellés et al., 2004), and have the non-specific proliferation potential for anaerobic bacteria (Ames et al.,

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1999). Meanwhile, positive effects on the growth and proliferation of *Lactobacilli* and *Bifidobacteria* were found using the MRPs formed from roasted bread and coffee beans (Borrelli and Fogliano, 2005; Jaquet et al., 2009). Nevertheless, *in vivo* information related to the effects of MRPs derived from soybean peptides on antioxidant capacity and gut microflora is still limited.

The percentage of people over 65 years old in China increased from 7% in 2010 to 11.4% in 2016, and many aged people are suffering from age-related diseases, which might lead to functional decline, cognitive dysfunctions, chronic diseases, and ultimately death (Lan et al., 2012). Although the aging biological mechanisms are still poorly understood, it is well recognized that aging is closely related to oxidative stress reactions and the formation of free radicals, which would lead to the metabolic abnormality of organs and destruction of genetic materials (Kumar et al., 2011; Stadtman, 2006). Meanwhile, the aging process usually is accompanied by gut microbial dysregulation, leading to a steady increase in malnutrition, constipation and colorectal cancer (Anand et al., 2012). Many studies have indicated that the intake of antioxidants, either from the daily diet or additional supplementation, is conducive to retarding aging rates, maintaining normal body metabolism and prolonging life span (Monente et al., 2015; Patrignani et al., 2016). Positive effects of ascorbic acid (Vitamin C, Vc) on natural killer (NK) cell activity in old mice and the reduction of oxidative stress by dietary supplementation have been reported (Ferrández et al., 1999; Lykkesfeldt et al., 1998). As one main protein-derived MRP in Chinese Shanxi aged vinegar, melanoidins have been found to activate Nrf2 transcription, then Nrf2-regulated genes, such as HO-1, NQO1 and GCS, would be expressed, and anti-aging system would be established via eliminating reactive oxygen species (ROS) (Forman, 2016; Xia et al., 2017). Human studies have shown that oligofructose could improve the gut bacterial profile in older adults (Naughton et al., 2011), and the probiotic function would be beneficial in antioxidative and anti-inflammatory effects *in vivo* (Yeh et al., 2014).

The D-galactose-induced aged mouse model has been widely utilized for anti-aging studies (Anand et al., 2012; Aydın et al., 2016), as the injection of D-galactose would accelerate the generation of ROS and advanced glycation end products (AGEs), which would simultaneously lead to behavioral and neurochemical changes similar to those of aging (Ho et al., 2003). In this study, the *in vivo* effects of consumption of MRPs from 1 to 3 kDa soybean peptides on aged-induced mice through subcutaneous injection of D-galactose were investigated. MRPF3 excretion was measured via urine fluorescence intensity and fecal color evaluations, while gut microbiota and antioxidant potential were analyzed.

2. Materials and methods

2.1. Preparation of MRPF3

Soybean peptides and MRPF3 were prepared according to our previous study (Yu et al., 2018). Soybean meal powder was hydrolyzed using alkaline protease and Flavourzyme in sequence, and 1–3 kDa peptides were obtained using ultrafiltration. The mixture of 1–3 kDa soybean peptides (1.5 g), L-cysteine (0.3 g) and D-xylose (0.6 g) was dissolved to a total volume of 20 ml with deionized water, and the pH of the solution was adjusted to 7.4 with 2 M NaOH. The solution was heated at 120 °C for 120 min with magnetic stirring at 800 r/min in a sealed vessel in an oil bath. Then, the MRPs were cooled quickly in ice water and lyophilized for further use.

2.2. Animals and treatments

According to a previous study (Sun et al., 2007), five-week old ICR male mice purchased from the Experimental Animal Center of Anhui Medical University, Anhui, China, were used in this research. After one week of adaptation, The ICR mice were randomly divided into seven groups (at least 10 animals per group). One group of animals was administered with an equal volume of saline solution and served as the control or normal growth group (group C), six groups were subcutaneously injected with D-galactose (Sigma-Aldrich Co., St. Louis, MO, USA) 1000 mg kg^{-1} mouse weight/day plus special sample for 5 weeks. According to the recommended minimum intake of commercial MRPs, three groups of D-galactose-treated mice were administered with MRPF3 at 800, 400 and 200 mg kg^{-1} mouse weight/day as high (group H), medium (group M) and low (group L) doses by gavage, respectively. Two groups of D-galactose-treated mice were given ascorbic acid (group Vc) and oligofructose (group D) at an intake of 400 mg kg^{-1} mouse weight/day as positive controls for the hydrogen atoms donation and gut probiotics mechanisms, respectively. The other remaining group of mice was given daily D-galactose plus an equal volume of saline solution as the aging model control group (group A). Each group was housed in a metabolic cage individually, and the animals were kept in an environmentally controlled room under standard conditions (12-h light and 12-h dark cycle and 55–70% humidity at room temperature) according to the regulations regarding experimental animals' administration published by Anhui province, China. The study was approved by the academic Animal Welfare Committee.

The animals were fed with different treatments for five weeks (experimental weeks), and body weights were monitored weekly. In the last day of the experimental period, animals were suffocated with CO_2, and blood samples were then collected into tubes by piercing the heart. The liver, kidney, thymus and spleen were removed, weighed and frozen. Then, the serum and 10% liver homogenate supernatants in 0.25 M sucrose solution were obtained by centrifugation at 12000 r/min for 10 min at 4 °C for further analyses. The intestines of the animals were also collected and stored at - 80 °C for the gut microbial experiments.

2.3. Determination of organ indexes

The organ indexes, including liver index, kidney index, thymus index and spleen index, were calculated by the equation: Organ index = Organ weight (mg)/body weight (g).

2.4. Fluorescence intensity of urine

Urine collection of each group was conducted on every Wednesday during the feeding treatment, and the urine samples were diluted 2-fold with deionized water and filtered through Whatman Filter Papers (No. 40, ashless, Whatman, England). The fluorescence intensity was measured by an F-7000 Hitachi fluorescence spectrometer (Tokyo, Japan) at an excitation wavelength of 347 nm and emission wavelength of 415 nm. The samples were placed in quartz glass cuvettes with a light path of 1 cm. Each sample was measured in triplicate.

2.5. Color changes of feces

The feces of each animal were collected on Saturday of each week and stored separately. After lyophilization, the color of each fecal sample was detected using a Chroma Meter (NR200 model, Shenzhen 3NH Technology Co., Ltd., Shenzhen, China), and the Hunter scale of L^* (from black (0) to white (100)), a^* (from green (negative) to red (positive)) and b^* (from blue (negative) to yellow (positive)) values were used as indicators.

2.6. Determination of gut microbial diversity

DNA from the intestinal contents (7 groups, at least 3 representative samples in each group) was extracted using the E.Z.N.A.® Stool DNA Kit (D4015, Omega, Inc., USA) for high-throughput community sequencing, and ultrapure water was used as a negative control to exclude the possibility of false-positive PCR results.

The V3—V4 region of the prokaryotic (bacterial and archaeal) small-subunit (16S) rRNA gene was amplified with slightly modified versions of primers of 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAT-3′) (Fadrosh et al., 2014). The 5′ ends of the primers were tagged with specific barcodes per sample and sequencing universal primers.

PCR amplification was performed in a 25 μL total volume reaction mixture containing 25 ng of template DNA, 12.5 μL PCR premix, 2.5 μL of each primer, and PCR-grade water. Standard PCR thermocycling for the prokaryotic 16S fragment amplification was 98 °C 30 s for an initial denaturation, 35 cycles of denaturation at 98 °C for 10 s, annealing at 54 °C/52 °C for 30 s, followed by extension at 72 °C for 45 s and final extension at 72 °C for 10 min. The PCR products were confirmed with 2% agarose gel electrophoresis, purified using AM Pure XT beads (Beckman Coulter Genomics, Danvers, MA, USA) and quantified using a Qubit Fluorometer (Invitrogen Co., Carlsbad, CA, USA).

The amplicon pools were prepared for sequencing, and the size and quantity of the amplicon library were assessed on an Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Santa Clara, USA). Multiplex pyrosequencing of samples was conducted on a 454 Roche GS-FLX Titanium instrument (Life Sciences Inc., Branford, CT, USA) according to the manufacturer's instructions.

Alpha diversity, including Chao1 (meaning community richness), Shannon (emphasizes the richness component of diversity), Simpson (emphasizes the evenness component of diversity) and Observed species (focuses species richness in samples) indexes, were calculated with the QIIME suite of programs (Version 1.8.0) (Lemos et al., 2011). Operational taxonomic units (OTUs) abundance was analyzed by rarefaction analysis (PyNAST software). Principal coordinate analysis (PCoA), analyzing differences at phylogenetic distances based on Unifrac analysis, was conducted with the QIIME suite of programs (Version 1.8.0). Dissimilarities in the individual microbial populations of different samples were compared by the MANOVA (multivariate analysis of variance) and Mann-Whitney test and executed in MATLAB R2011b (The Mathworks Inc., Natick, MA, USA).

2.7. In vivo antioxidant activities

Serum samples were obtained by centrifugation at 3500g for 15 min at 4 °C from the blood samples. The total antioxidant capacity (T-AOC) of serum, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in liver, and the content of malondialdehyde (MDA) in liver were detected using related kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, JiangSu, China) following the recommended procedures.

2.8. Statistical analysis

At least three repetitions were conducted in each experiment, and all data were statistically tested by one-way analysis of variance (ANOVA) using the SPSS software (version 13.0, SPSS Inc., Chicago, IL, USA). A P-value < 0.05 was considered a statistically significant difference.

3. Results

3.1. Determination of body weight

As shown in Table 1, body weight of each group remained stable ($P > 0.05$) at the end of the adaption week, but significant differences ($P < 0.05$) were found during the experiment weeks (1st to 5th week) with the increasing trends. Furthermore, at the end of the experiment weeks (5th week), the control group (group C) and group fed the ascorbic acid diet (group Vc) showed the highest body weights of 44.63 ± 1.60 g and 43.45 ± 1.78 g, respectively, followed by the weights of the medium-, low- and high-dose groups treated with MRPF3 for 42.81 ± 2.90 g, 40.96 ± 4.02 g and 39.21 ± 2.41 g, respectively, which were significantly higher than those in groups A and D.

3.2. Measurement of organ indexes

The organ indexes of different groups are shown in Table 2. The liver index of six groups did not differ ($P > 0.05$) with respect to the control group (group C). The lowest values of both thymus index and spleen index were found in the aging (group A) and oligofructose (group D) groups, which were significantly lower ($P < 0.05$) than the control (group C) and ascorbic acid (group Vc) groups. Compared with group A, the thymus index and spleen index in animals administered with MRPF3 (groups H, M and L) were found to be significantly increased. In addition, a decrease in the kidney index was obtained in the high-dose group (group H) treated with MRPF3 compared with the other groups.

3.3. Fluorescence intensity of urine

As shown in Table 3, no significant differences were observed in urine fluorescence intensity of each group during the adaptation week, but sharp increases were detected in the groups treated with MRPF3 (groups H, M and L) from the 1st experiment week. At the 5th experiment week, compared with the control group, although the urine fluorescence intensities in the Vc, oligofructose (group D) and aging (group A) groups did not show a difference, the values in the H, M and L groups were increased by 1.01, 0.59 and 0.50 times, respectively.

3.4. Measurement of color in feces

The fecal color in seven groups during the experimental period could be found in Table 4. The L^* values of feces in all groups significantly decreased, but the a^* and b^* values markedly increased from the 1st to 3rd experimental week. At the end of the experiment weeks, the fecal samples in the MRPF3 groups (groups H, M and L) showed lower L^* and higher a^*, b^* values than the other four groups, indicating a much more dark color.

3.5. Gut microbial analysis

3.5.1. Alpha diversity analysis

Alpha diversity generally includes observed species, Shannon, Chao1 and Simpson. As shown in Table 5, compared with the control (group C), the alpha diversity in all groups was decreased, and the lowest values of observed species, Shannon, Simpson, and Chao1 were all found in the aging group (group A), confirming the diversity reduction of in aging gut microbiota. Obvious increases of alpha diversity could be found in groups fed with MRPF3; and compared with the values in group A, the observed species in groups H, M and L increased by 15.19%, 8.50% and 7.9%, Shannon was 5.93%, 2.13% and 11.91% higher, Simpson was 6.07%, 4.26% and 10.94% higher, and Chao1 increased by 4.24%, 2.13% and 8.24% times, respectively. Meanwhile, the four indexes in group M were very similar to those in the oligofructose group (group D).

3.5.2. Microbial composition of different samples

At the phylum level, a total of 17 kinds of microorganisms were detected in all samples (Fig. 1a). The members of *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Bacteria unclassified*, *Actinobacteria* and *Candidatus Saccharibacteria* were in relatively greater abundances, accounting for more than 98.16% of the total number. It was clearly found that *Firmicutes* and *Bacteroidetes* were the most prevalent microbial taxa in all sample groups, which accounted for 53.83% and 34.96% of the total sequence reads, respectively. In addition, the gut microbial composition at the phylum level was variable in the different groups (Fig. 1a). Compared with the control group (group C), the abundance of

Table 1
Measurement of body weight in mice fed with different diets during the experimental period.

Groups	Body weight (g)					
	Initial weight	1st week	2nd week	3rd week	4th week	5th week
C	20.21 ± 1.07^{aE}	28.43 ± 1.42^{aD}	35.81 ± 1.70^{aC}	41.53 ± 2.58^{aB}	42.24 ± 1.73^{aB}	44.63 ± 1.60^{aA}
Vc	20.24 ± 1.50^{aF}	27.47 ± 1.79^{aE}	34.85 ± 3.25^{aD}	37.69 ± 3.08^{bC}	41.09 ± 1.87^{aB}	43.45 ± 1.78^{aA}
D	20.20 ± 1.61^{aD}	27.51 ± 1.53^{aC}	32.56 ± 2.57^{bcB}	34.78 ± 2.92^{cA}	36.17 ± 2.00^{cA}	36.55 ± 1.88^{dA}
A	20.72 ± 1.77^{aD}	27.81 ± 2.12^{aC}	31.74 ± 2.19^{cB}	34.15 ± 3.15^{cA}	35.75 ± 1.64^{cA}	35.43 ± 1.58^{dA}
H	20.93 ± 1.17^{aE}	26.94 ± 1.65^{aD}	31.86 ± 2.22^{cC}	35.70 ± 2.71^{bcB}	38.52 ± 3.76^{bA}	39.21 ± 2.41^{cA}
M	20.54 ± 1.23^{aF}	27.75 ± 0.91^{aE}	34.16 ± 2.08^{abD}	36.82 ± 3.23^{bcC}	40.18 ± 3.68^{abB}	42.81 ± 2.90^{abA}
L	20.03 ± 2.43^{aF}	27.83 ± 1.48^{aE}	32.26 ± 2.19^{bcD}	35.83 ± 1.33^{bcC}	38.73 ± 1.54^{bB}	40.96 ± 4.02^{bcA}

In order to avoid the experimental error resulting from animal individual differences, the truncated mean (or trimmed mean) was applied by removing the highest and lowest body weights prior to the average. Results are expressed as the mean ± standard deviation ($n = 8$). Values followed by different lowercase letters in the same column mean statistically significant differences ($P < 0.05$) among different treated groups. Values followed by different capital letters in the same line mean significantly different ($P < 0.05$) in a same group among different experimental weeks. C - control group, Vc - ascorbic acid group, D - oligofructose group, A - aging group, H - high-dose MRPF3 group, M - medium-dose MRPF3 group, L - low-dose MRPF3 group.

Table 2
Liver, spleen, thymus and kidney index analyses in mice fed with different diets.

Groups	Organs indexes in mice(mg organ/g body weight)				
	Liver	Spleen	Thymus	Kidney(left)	Kidney(right)
C	51.27 ± 1.94^{a}	5.04 ± 0.44^{a}	3.75 ± 0.44^{a}	10.08 ± 1.02^{a}	8.89 ± 0.76^{a}
Vc	50.13 ± 1.40^{a}	4.64 ± 0.81^{ab}	3.59 ± 0.56^{a}	9.96 ± 1.28^{a}	8.64 ± 0.86^{a}
D	48.70 ± 2.71^{a}	3.35 ± 0.57^{bc}	2.56 ± 0.48^{bc}	9.30 ± 1.02^{ab}	8.21 ± 0.80^{ab}
A	47.70 ± 1.03^{a}	3.25 ± 0.79^{c}	2.21 ± 0.29^{c}	9.02 ± 0.50^{ab}	8.35 ± 0.66^{ab}
H	49.70 ± 1.91^{a}	4.03 ± 0.52^{abc}	3.03 ± 0.48^{abc}	8.05 ± 0.37^{b}	7.25 ± 0.29^{b}
M	49.29 ± 1.36^{a}	4.27 ± 0.48^{abc}	3.33 ± 0.60^{ab}	9.51 ± 1.42^{ab}	8.52 ± 0.86^{ab}
L	49.56 ± 2.10^{a}	3.98 ± 0.93^{abc}	2.93 ± 0.42^{abc}	9.70 ± 0.66^{ab}	8.54 ± 0.13^{ab}

In order to avoid the experimental error resulting from animal individual differences, the truncated mean (or trimmed mean) was applied by removing the highest and lowest organs indexes prior to the average. Results are expressed as the mean ± standard deviation ($n = 8$). Different lowercase letters within a column indicate significant differences ($P < 0.05$) among different treated groups. C - control group, Vc - ascorbic acid group, D - oligofructose group, A - aging group, H - high-dose MRPF3 group, M - medium-dose MRPF3 group, L - low-dose MRPF3 group.

Table 3
Determination of urine fluorescence intensity in mice fed with different diets during the experimental period.

Groups	Fluorescence intensity of urine				
	1st week	2nd week	3rd week	4th week	5th week
C	188.05 ± 19.58^{aA}	209.83 ± 15.21^{cA}	226.51 ± 16.10^{dA}	227.92 ± 25.92^{cA}	231.25 ± 31.23^{cA}
Vc	186.24 ± 22.30^{aC}	208.31 ± 24.75^{cBC}	236.15 ± 18.81^{dAB}	248.80 ± 8.93^{cAB}	253.74 ± 32.03^{cA}
D	188.85 ± 16.10^{aA}	202.09 ± 12.13^{cA}	208.25 ± 8.40^{aA}	232.09 ± 10.28^{cA}	248.32 ± 24.85^{cA}
A	185.84 ± 25.06^{aB}	204.95 ± 15.48^{cAB}	217.38 ± 23.88^{dAB}	250.35 ± 39.35^{cA}	255.46 ± 41.31^{cA}
H	188.40 ± 8.72^{aC}	357.22 ± 17.91^{aB}	431.75 ± 27.18^{aA}	457.92 ± 28.63^{aA}	465.49 ± 47.24^{aA}
M	184.49 ± 25.59^{aC}	260.42 ± 28.46^{bB}	330.01 ± 27.39^{bA}	360.52 ± 30.71^{bA}	366.61 ± 41.98^{bA}
L	182.32 ± 18.89^{aC}	246.03 ± 11.15^{bB}	281.02 ± 26.88^{cB}	329.83 ± 12.98^{bA}	347.90 ± 44.58^{bA}

In order to avoid the experimental error resulting from animal individual differences, the truncated mean (or trimmed mean) was applied by removing the highest and lowest fluorescence intensities prior to the average. Results are expressed as the mean ± standard deviation ($n = 8$). Values followed by different lowercase letters in the same column mean statistically significant differences ($P < 0.05$) among different treated groups. Values followed by different capital letters in the same line mean significantly different ($P < 0.05$) in the same group among different experimental weeks. C - control group, Vc - ascorbic acid group, D - oligofructose group, A - aging group, H - high-dose MRPF3 group, M - medium-dose MRPF3 group, L - low-dose MRPF3 group.

Firmicutes in the aging group (group A) was significantly decreased, while the *Bacteroidetes* was present in a higher abundance. However, the higher abundance of *Firmicutes* with lower abundance of *Bacteroidetes* were detected when the animals were fed with MRPF3 (groups H, M and L) or oligofructose (group D) for five weeks, and *Firmicutes* had become the most prevalent microorganism. The ratios of *Firmicutes* to *Bacteroides* in the different groups were as follows: group D > group Vc > group H > group M > group C > group L > group A. Furthermore, the relative abundance of potential pathogenic bacteria in groups H, M and L were all significantly lower than those in groups C and A.

Microbial composition of different samples at the family level was shown in Fig. 1b. The relative abundances of *Porphyromonadaceae*, *Lactobacillaceae*, *Lachnospiraceae*, *Prevotellaceae*, *Ruminococcaceae*, *Bacteroidaceae*, *Clostridiales unclassified*, and *Helicobacteraceae* were found to be higher in all samples. In addition, it is obvious that *Lactobacillaceae* was predominant in the groups treated with MRPF3, and the abundances of *Lactobacillaceae* in groups H (31.36), M (24.52) and L (45.45) were higher than that in the group fed with oligofructose (20.62). Compared with group A, the relative abundance of *Lachnospiraceae* was decreased by 50.11%, 31.73%, 96.65% and 59.31% in groups H, M, L and D, respectively, while *Porphyromonadaceae* in group A was much higher than that in the other six groups.

3.5.3. Unifrac analysis

Multivariate analysis was used to comparatively analyze the

Table 4
Fecal color changes in mice fed with different diets during the experimental period.

time (week)		Color of feces in different groups						
		C	Vc	D	A	H	M	L
1st	L^*	61.83 ± 1.59^{aA}	62.82 ± 3.36^{aA}	62.50 ± 5.59^{aA}	63.28 ± 0.63^{aA}	62.20 ± 1.33^{aA}	62.61 ± 1.78^{aA}	62.55 ± 4.04^{aA}
	a^*	1.80 ± 0.40^{aB}	1.90 ± 0.07^{aB}	1.92 ± 0.31^{aA}	2.17 ± 0.43^{aA}	1.94 ± 0.30^{aC}	1.97 ± 0.47^{aB}	1.94 ± 0.05^{aB}
	b^*	8.05 ± 0.67^{aC}	8.28 ± 1.56^{aA}	8.08 ± 1.93^{aA}	8.62 ± 1.82^{aA}	8.80 ± 1.37^{aB}	7.87 ± 1.75^{aA}	8.32 ± 1.12^{aB}
2nd	L^*	57.54 ± 2.29^{aB}	54.98 ± 3.94^{aB}	57.71 ± 2.83^{aBC}	55.23 ± 0.92^{aB}	53.44 ± 2.14^{aB}	55.99 ± 4.10^{aB}	54.80 ± 2.53^{aB}
	a^*	2.26 ± 0.55b^{abAB}	2.52 ± 0.17^{abAB}	2.54 ± 0.43^{abA}	1.93 ± 0.49^{bAB}	3.06 ± 0.12^{aB}	3.00 ± 0.17^{aAB}	2.86 ± 0.35^{bB}
	b^*	10.16 ± 1.16^{aB}	10.93 ± 1.31^{aAB}	12.73 ± 0.99^{aAB}	12.87 ± 2.94^{aA}	11.92 ± 1.62^{aB}	11.51 ± 2.11^{aAB}	11.45 ± 2.75^{aA}
3rd	L^*	53.61 ± 1.92^{aC}	51.90 ± 1.29^{aB}	53.85 ± 3.35^{aC}	53.40 ± 2.17^{aBC}	45.04 ± 1.65^{bC}	47.24 ± 1.84^{bC}	48.06 ± 1.81^{aBC}
	a^*	2.70 ± 0.03^{cdA}	3.08 ± 0.49^{bcdA}	2.20 ± 0.22^{dA}	2.39 ± 0.19^{cdAB}	4.41 ± 1.04^{aA}	4.19 ± 1.02^{abA}	3.89 ± 0.63^{abcA}
	b^*	12.82 ± 0.96^{abA}	11.89 ± 1.99^{abA}	10.26 ± 1.05^{bA}	12.29 ± 2.17^{abA}	15.16 ± 2.29^{aA}	13.87 ± 2.48^{abA}	13.64 ± 3.04^{abA}
4th	L^*	51.31 ± 1.12^{abC}	52.44 ± 1.28^{aB}	54.97 ± 2.79^{aC}	51.38 ± 0.58^{abC}	44.65 ± 5.01^{cC}	46.20 ± 3.69^{bcC}	47.76 ± 1.93^{abBC}
	a^*	2.87 ± 0.26^{bcA}	3.56 ± 1.14^{cA}	2.44 ± 0.47^{abcA}	2.43 ± 0.54^{cAB}	4.49 ± 0.50^{aA}	3.74 ± 0.59^{abA}	3.93 ± 0.58^{abcA}
	b^*	12.98 ± 1.05^{abA}	11.78 ± 1.63^{bA}	13.35 ± 1.99^{abA}	12.37 ± 2.57^{abA}	15.65 ± 0.55^{aA}	13.16 ± 2.27^{abA}	13.53 ± 2.17^{abA}
5th	L^*	51.24 ± 3.13^{abC}	51.77 ± 4.56^{abB}	55.08 ± 3.84^{aC}	52.28 ± 2.35^{aC}	44.27 ± 1.70^{cC}	45.26 ± 2.65^{bcC}	47.10 ± 5.27^{abcC}
	a^*	2.72 ± 0.48^{cA}	3.34 ± 0.56 ^{bcA}	2.57 ± 0.45^{cA}	2.75 ± 0.08^{cA}	4.72 ± 0.54^{aA}	3.89 ± 0.64^{abA}	3.33 ± 0.37^{bcA}
	b^*	13.06 ± 1.31^{aA}	12.77 ± 1.80^{aA}	11.84 ± 2.00^{aA}	12.75 ± 2.30^{aA}	15.76 ± 2.36^{aA}	13.54 ± 2.67^{aA}	14.02 ± 2.33^{aA}

In order to avoid the experimental error resulting from animal individual differences, the truncated mean (or trimmed mean) was applied by removing the highest and lowest color values prior to the average. Results are expressed as the mean ± standard deviation ($n = 8$). Values followed by different lowercase letters in the same column mean statistically significant differences ($P < 0.05$) among different treated groups. Values followed by different capital letters in the same line mean significantly different ($P < 0.05$) in the same group among different experimental weeks. C - control group, Vc - ascorbic acid group, D - oligofructose group, A - aging group, H - high-dose MRPF3 group, M - medium-dose MRPF3 group, L - low-dose MRPF3 group.

Table 5
Summary of observed species, Chao1, Shannon and Simpson index in mice fed with different diets.

Samples	Observed species	Shannon	Simpson	Chao1
C	1121	8.03	0.99	1446.66
Vc	982	7.57	0.98	1267.44
D	1016	7.50	0.96	1311.94
A	882	7.08	0.94	1172.31
H	1044	7.35	0.95	1424.13
M	957	7.51	0.98	1300.52
L	952	7.38	0.96	1268.93

C - control group, Vc - ascorbic acid group, D - oligofructose group, A - aging group, H - high-dose MRPF3 group, M - medium-dose MRPF3 group, L - low-dose MRPF3 group.

bacterial microbiota among the seven groups. PCoA based on weighting is shown as Fig. 2a with PC1 and PC2 accounting for 41.16% and 32.35.9% of the total variance, and Fig. 2b represents unweighted versions of the Unifrac distance metric with PC1 and PC2 representing 27.02% and 22.87% of the total variance, respectively. As indicated in Fig. 2a, groups H, M and L were closer to the oligofructose group (group D), which indicated the homology in bacterial microbiota. Meanwhile, all the four groups were far from group A, indicating the differences between these groups and aging group in the bacterial microbiota.

Similar results could be found in Fig. 2b; the adjacency among groups H, M and D revealed the similar composition of bacterial microbiota, and all the three groups were close to the control (group C) group, indicating the bacterial microbiota of the four groups were analogous.

3.6. *In vivo* antioxidant activity

The T-AOC in serum, GSH-Px, SOD activities and MDA content in the livers of the different groups are shown in Fig. 3. As presented in Fig. 3a, a significant decrease ($P < 0.05$) of the T-AOC was detected in the D-galactose-treated groups compared with the control (group C), and the lowest T-AOC was observed in the aging (group A) and oligofructose (group D) groups. The T-AOC was significantly increased by treatment with MRPF3, and the T-AOC in groups H, M and L was 50.99%, 64.79% and 37.81% higher than that in group A, respectively.

Similarly, for the GSH-Px and SOD activities, the highest values were observed in groups C and Vc, followed by groups M, H and L, while the lowest GSH-Px and SOD activities were observed in the aging group (group A) (Fig. 3b and c). In contrast, the highest MDA content was observed in group A (5.70 U/ml), and the lowest was detected in group C (2.37 U/ml) (Fig. 3d). Significant decreases ($P < 0.05$) of MDA content were observed in the groups treated with Vc and MRPF3 (groups H, M and L) with the values of 2.98, 3.22, 3.33, 3.89. 4.26 U/ml, respectively.

4. Discussion

D-galactose-injected rodent models have been widely utilized in aging studies, and the high-dose injection of D-galactose for one week would lead to the expression of galactitol and/or hydrogen peroxide, then they in turn caused the metabolism of sugar and ROS in disorder and resulted in the forming of aging model (Anand et al., 2012; Aydın et al., 2016; Shen et al., 2002). Nevertheless, inconsistency of experimental results obtained by the animal model is very common, because of the diversity of administration dose of D-galactose, animal strain and administration duration (Sadigh-Eteghad et al., 2017).

During the experimental period, the lowest body weight was obtained in the group A (Table 1) could be explained by oxidative injury induced by D-galactose injection (Lu et al., 2007). A significant increase in body weight was found in the animal group treated with Vc, as the antioxidant could repair oxidative damage induced by D-galactose (Sönmez et al., 2005). However, the intake of the oligofructose (group D) seemed to have little effect on the weight loss, which might be due to the hysteretic nature of probiotic function. It should be noticed that MRPF3 (groups H, M and L) significantly ameliorated the weight loss in aging models, as MRPs have been confirmed as effective free radical scavengers to protective against oxidative deterioration (Patrignani et al., 2016; Yu et al., 2018).

As shown in Table 2, the liver indexes in the seven groups did not differ significantly ($P > 0.05$), however, thymus and spleen indexes were significantly decreased in the groups treated with D-galactose. Previous studies have found that the aging induced by D-galactose could result in the degradation and reduction of immune organs (Liu et al., 2018). In addition, compared with group C, the spleen and thymus indexes in the groups treated with MRPF3 (groups H, M and L) did not differ significantly, suggesting that MRPF3 might protect the organs from oxidative damage and enhance the immunity of the mice

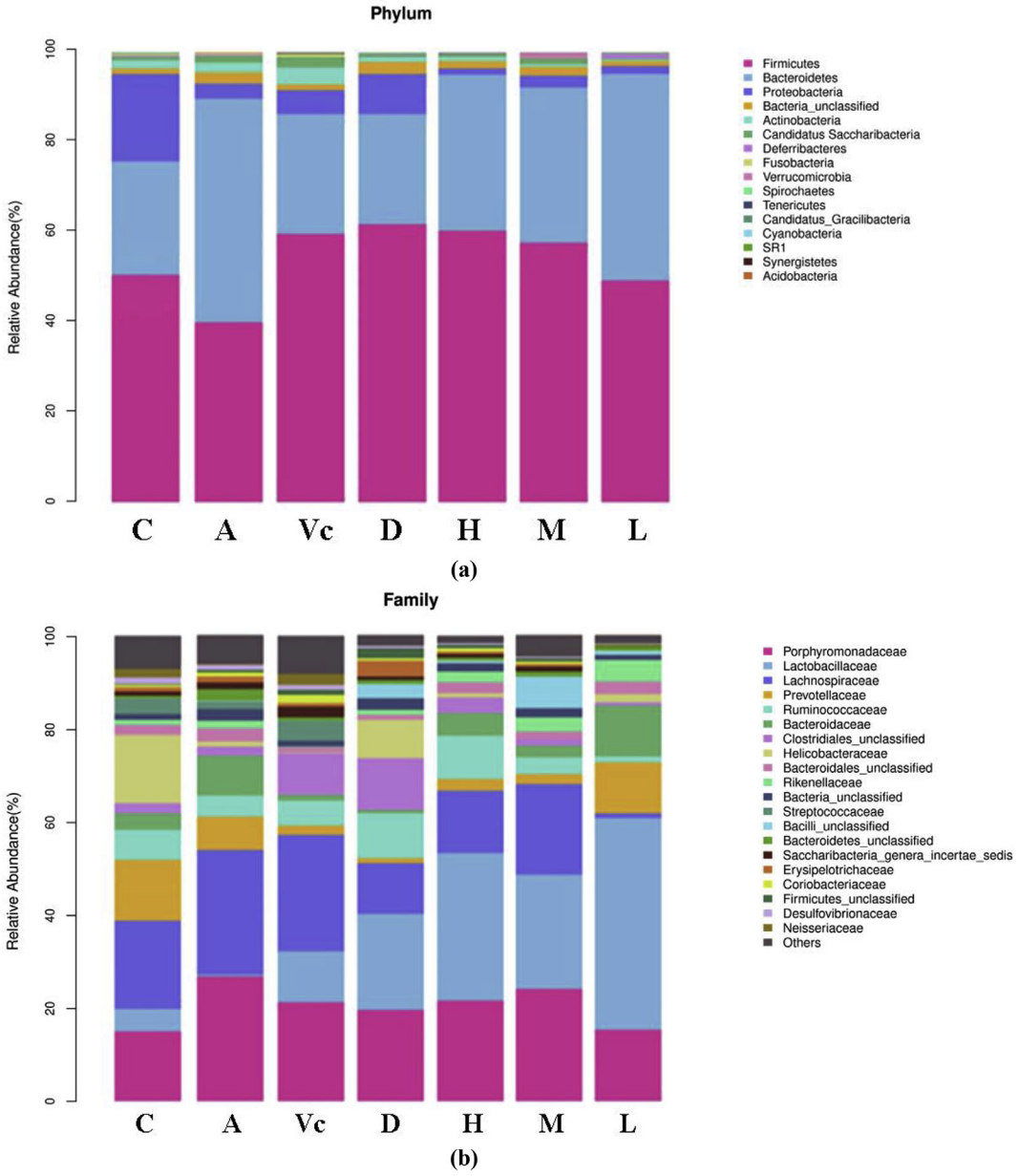

Fig. 1. Composition and relative abundances of bacterial communities based on 16S rDNA sequence analysis in different groups (C - control group, A - aging group, Vc - ascorbic acid group, D - oligofructose group, H - high-dose MRPF3 group, M - medium-dose MRPF3 group, and L - low-dose MRPF3 group), (a) at the phylum level, and (b) at the family level.

(Patrignani et al., 2016). It is noteworthy that the kidney index in the group treated with a high-dose of MRPF3 (group H) was much lower than other groups. According to a previous study, kidney growth and development may be affected by a high dose of MRPs (Tuohy et al., 2006), but little *in vivo* and clinical data have been reported that a high dose dietary intake of MRPs could increase the incidence of kidney disease yet.

Fluorescent compounds developed during the MR have been proven to be the precursors of the brown pigments and considered as early indicators of the MR (Wu et al., 2014). In Table 3, a substantial increase of urine fluorescence intensity was detected in the MRPF3-treated animals (groups H, M and L), suggesting that more AGEs were ingested, and the AGEs obtained in MR, e.g. pentosidine, would not be proteolysis or breakdown, would result in a higher fluorescence intensity through urine enrichment (Förster et al., 2005). Furthermore, it has been reported that the intake of high level AGEs would increase the potential risk to form toxic AGEs through reactions with serum or tissue components, however, the toxic and side effects were not well-documented yet (Nguyen, 2006).

As shown in Table 4, the color of the feces became darker with the increases in feeding time, which may be related to the changes in species and numbers of intestinal microorganisms as well as the content of bile pigments during the animals' growth (Jiménez-Escrig et al., 2013). Moreover, the fecal color was significantly darker ($P < 0.05$) in the group treated with MRPF3, which could be explained by the poor digestibility of Maillard browning products formed via the cross-linking reactions of proteins (or peptides) and sugars (or peptides) (Delgadoandrade et al., 2013).

Alpha diversity is commonly used to express the abundance of species in community ecology, and it is a comprehensive indicator of species richness and/or evenness (Kemp and Aller, 2004). The decrease of biodiversity and compromised stability in the gut microbiota have been reported in elderly people (Tropek et al., 2016), which is similar with our results in aging mice (Fig. 1 and Table 5). A sharp increase in

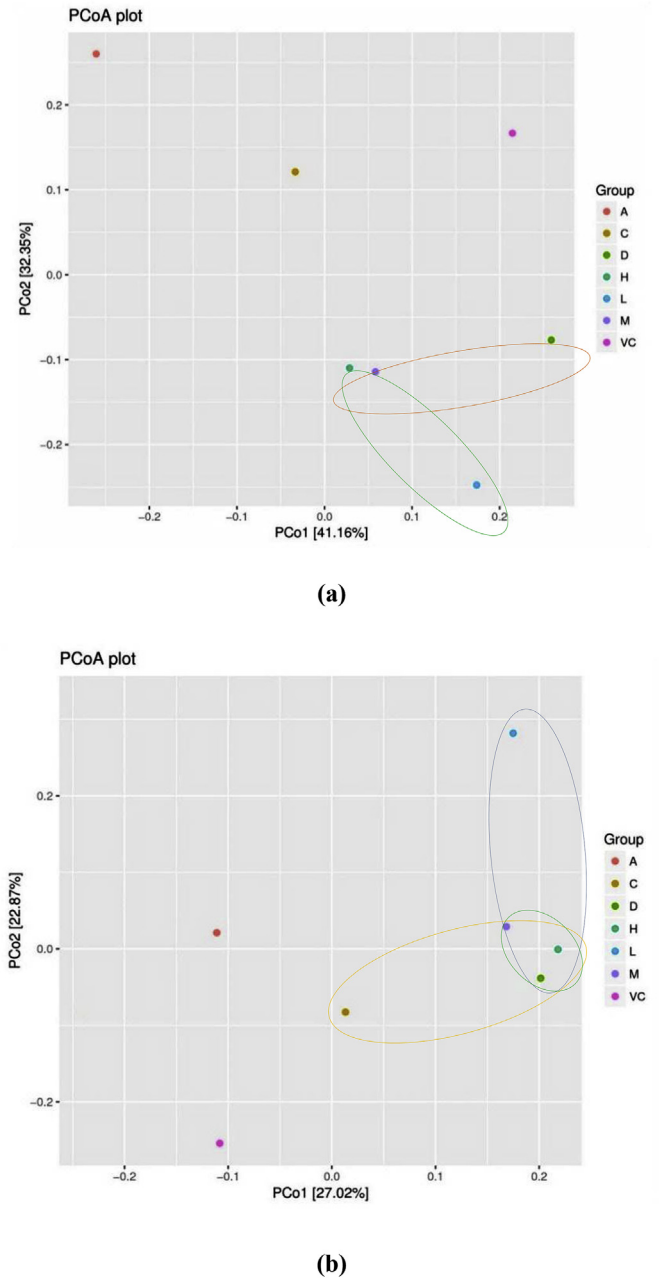

Fig. 2. Principal coordinate plots (PCoA) generated using the Unifrac algorithm for individual sample data set. A, C, D, H, M, L and Vc represent the aging group, control group, oligofructose group, high-dose MRPF3 group, medium-dose MRPF3 group, low-dose MRPF3 group and ascorbic acid group, respectively. (a) PCoA based on weighted and (b) PCoA based on unweighted versions of the Unifrac distance metric.

species was observed when the mice treated with MRPF3 (groups H, M and L), and the four indexes in the medium-dose group (group M) were similar to those in the oligofructose group, indicating that MRPs prepared from 1 to 3 kDa soybean peptides could remedy the reduction of gut microbial diversity caused by aging.

The gut microbiota can be inevitably affected by age, modification of diet and immune system of the host (Woodmansey, 2007). As shown in Fig. 1a and b, the abundance ratio of *Bacteroidetes* and *Firmicutes* was significantly reduced in the aging group (group A). Similar to our result, previous research reported that the abundance of *Firmicutes* was much higher in the gut of the young, and *Bacteroidetes* was the most prevalent microbial type in aging samples (Claesson et al., 2011). Oligofructose has been widely recognized as one of the prebiotics and often used to selectively stimulate the activity and growth of *Bifidobacteria* and *Lactobacilli* in the gut (Saulnier et al., 2007). Moreover, the highest abundances of *Lactobacillaceae* were detected in the MRPF3 groups, which were higher than that in the oligofructose group. Since the melanoidins cannot be digested in the upper gastrointestinal tract, it has been proven that they would selectively enhance the proliferation of beneficial bacteria (Borrelli and Fogliano, 2005). Contrary to the situation of *Lactobacilli*, the abundances of *Porphyromonadaceae* and *Lachnospiraceae* in groups treated with MRPF3 were much lower than in group A. As anaerobic bacteria, *Porphyromonadaceae* and *Lachnospiraceae* were associated with many diseases, such as inflammatory bowel disease (IBD) and cirrhosis (Giannelli et al., 2014). It has been reported that the relative abundances of *Porphyromonadaceae* and *Lachnospiraceae* were much higher in the gut of colorectal patients, and a significant increase of *Porphyromonadaceae* was also found in aged mice (Zackular et al., 2014).

In addition, similar gut microbial composition in the MRPF3 groups and the oligofructose group were observed from the PCoA results (Fig. 2). Previous studies have shown that the MRPs from bread crust could be used as the potential prebiotics metabolized efficiently by beneficial bacteria as carbon or nitrogen sources *in vitro* (Borrelli and Fogliano, 2005). Similarly, melanoidins derived from biscuits could also increase the lactic bacteria count and ratio between lactic and total aerobic bacteria *in vivo* (Patrignani et al., 2016).

As shown in Fig. 3, the highest contents of MDA but lowest activities of SOD, GSH-Px as well as T-AOC were found in the aging group. MDA is a water-soluble secondary product of lipid peroxidation, and it is widely used as an indicator of lipid peroxidation (Sirivarasai et al., 2015). Antioxidant enzyme systems, including GSH-Px, SOD, etc., are important in preventing the formation of ROS and protecting the cells from the damages caused by free radicals (Pan et al., 2016). Nevertheless, the activities of SOD, GSH-Px as well as T-AOC were significantly improved, and MDA content was decreased significantly, when animals were fed with MRPF3, which was consistent with the results obtained by feeding the mice using the MRPs derived from biscuits (Patrignani et al., 2016). Similarly, it was also found that the content of GSH-Px increased by 16% upon coffee consumption for 1 week in the human body (Somoza, 2005). Furthermore, as important participants in metabolism, it has been suggested that the gut microbes have significant impacts on human immune system (Dodd et al., 2017; Strober, 2010). Then, the antioxidant potential of the middle-dose MRPF3 group (group M) would be predominant by inhibition of the growth of pathogenic bacteria and promoting the proliferation of beneficial bacteria.

5. Conclusions

In conclusion, the consumption of these specific MRPF3 could retard aging progress by enhancing *in vivo* antioxidant capacity, regulating gut microbes and protecting immune organs from free radical damages. In addition, the diet of MRPF3 could be excreted through the urine and feces, leading to increases in fecal color as well as urine fluorescence intensity.

Conflicts of interest statement

The authors declare no competing financial interest.

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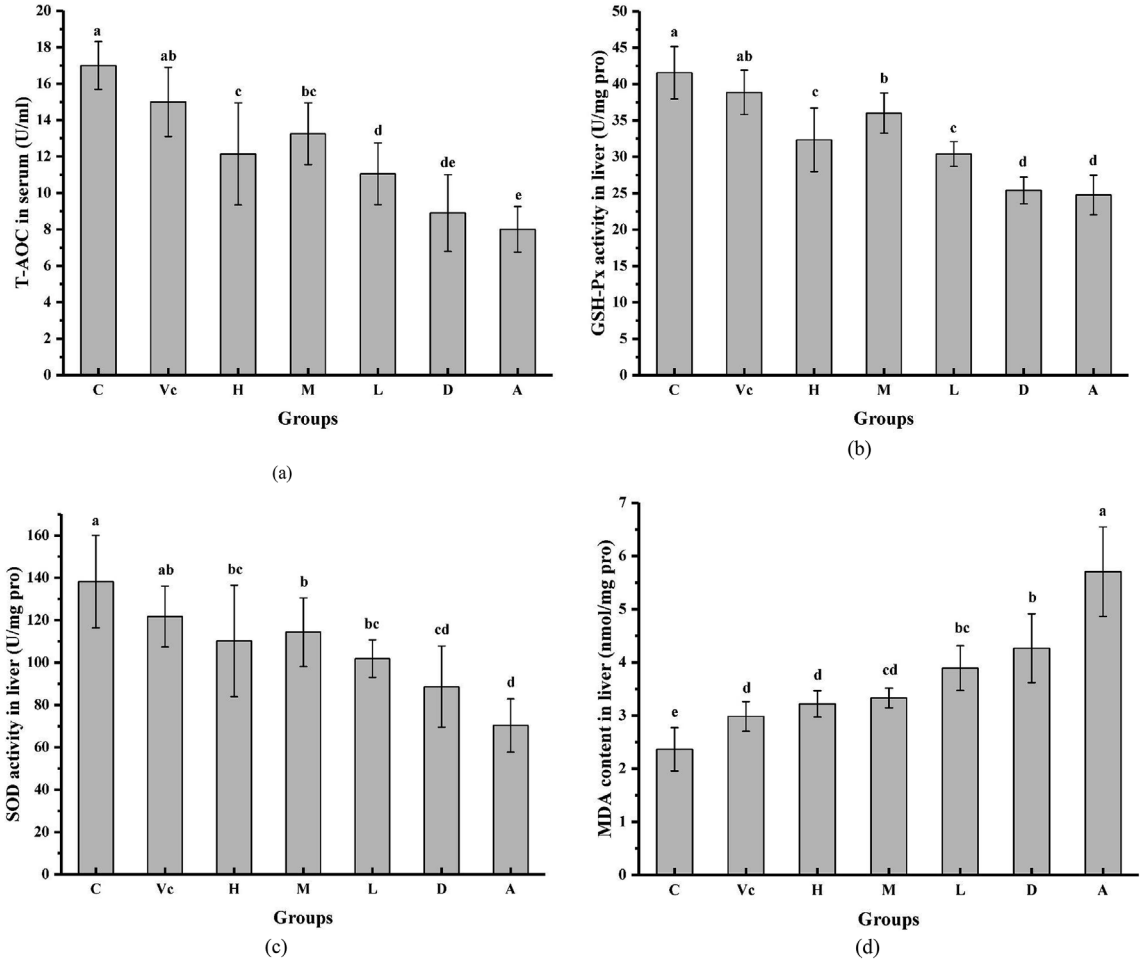

Fig. 3. *In vivo* antioxidant capacity determination of different groups (C - control group, Vc - ascorbic acid group, H - high-dose MRPF3 group, M - medium-dose MRPF3 group, L - low-dose MRPF3 group, D - oligofructose group and A - aging group). (a) T-AOC in serum, (b) GSH-Px activity in liver, (c) SOD activity in liver and (d) MDA content in liver. Different superscript letters indicate significant differences at $P < 0.05$.

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References

Ames, J.M., Wynne, A., Hofmann, A., Plos, S., Gibson, G.R., 1999. The effect of a model melanoidin mixture on faecal bacterial populations *in vitro*. Br. J. Nutr. 82, 489–495.
Anand, K.V., Mohamed Jaabir, M.S., Thomas, P.A., Geraldine, P., 2012. Protective role of chrysin against oxidative stress in D-galactose-induced aging in an experimental rat model. Geriatr. Gerontol. Int. 12, 741–750.
Aydın, A.F., J, Ç., Doğanekici, I., Betülkalaz, E., Doğruabbasoğlu, S., Uysal, M., 2016. Carnosine and taurine treatments diminished brain oxidative stress and apoptosis in D-galactose aging model. Metab. Brain Dis. 31, 337–345.
Borrelli, R.C., Fogliano, V., 2005. Bread crust melanoidins as potential prebiotic ingredients. Mol. Nutr. Food Res. 49, 673–678.
Claesson, M.J., Cusack, S., O'Sullivan, O., Greenediniz, R., Weerd, H.D., Flannery, E., Marchesi, J.R., Falush, D., Dinan, T., Fitzgerald, G., 2011. Composition, variability, and temporal stability of the intestinal microbiota of the elderly. P. Nat. Acad. Sci. USA 108, 4586–4591.
Delgadoandrade, C., Ronceroramos, I., Haro, A., Morales, F.J., Seiquer, I., Navarro, M.P., 2013. Effects of diets supplemented with MRPs from bread crust on the food intake and body weights in rats. Food Funct 4, 1016–1022.
Dodd, D., Spitzer, M.H., Van, T.W., Merrill, B.D., Hryckowian, A.J., Higginbottom, S.K., Le, A., Cowan, T.M., Nolan, G.P., Fischbach, M.A., 2017. A gut bacterial pathway metabolizes aromatic amino acids into nine circulating metabolites. Nature 3, e00438.
Domínguez, R., Gómez, M., Fonseca, S., Lorenzo, J.M., 2014. Effect of different cooking methods on lipid oxidation and formation of volatile compounds in foal meat. Meat Sci. 97, 223–230.
Fadrosh, D.W., Bing, M., Gajer, P., Sengamalay, N., Ott, S., Brotman, R.M., Ravel, J., 2014. An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. Microbiome 2, 6.
Ferrández, M., Correa, R., Del Rio, M., De la Fuente, M., 1999. Effects *in vitro* of several antioxidants on the natural killer function of aging mice. Exp. Gerontol. 34, 675–685.
Forman, H.J., 2016. Redox signaling: an evolution from free radicals to aging. Free Radical Biol. Med. 97, 398–407.
Förster, A., Kühne, Y., Henle, T., 2005. Studies on absorption and elimination of dietary maillard reaction products. Ann. NY Acad. Sci. 1043, 474–481.
Giannelli, V., Gregorio, V.D., Iebba, V., Giusto, M., Schippa, S., Merli, M., Thalheimer, U., 2014. Microbiota and the gut-liver axis: bacterial translocation, inflammation and infection in cirrhosis. World J. Gastroenterol. 20, 16795–16810.
Ho, S.C., Liu, J.H., Wu, R.Y., 2003. Establishment of the mimetic aging effect in mice caused by D-galactose. Biogerontology 4, 15–18.
Jaeger, H., Janositz, A., Knorr, D., 2010. The Maillard reaction and its control during food processing. The potential of emerging technologies. Pathol. Biol. 58, 207–213.
Jaquet, M., Rochat, I., Moulin, J., Cavin, C., Bibiloni, R., 2009. Impact of coffee consumption on the gut microbiota: a human volunteer study[J]. Int. J. Food Microbiol. 130, 117–121.
Jiménez-Escrig, A., Gómez-Ordóñez, E., Tenorio, M.D., Rupérez, P., 2013. Antioxidant and prebiotic effects of dietary fiber co-travelers from sugar Kombu in healthy rats. J.

Appl. Phycol. 25, 503–512.
Kemp, P.F., Aller, J.Y., 2004. Bacterial diversity in aquatic and other environments: what 16S rDNA libraries can tell us. FEMS Microbiol. Ecol. 47, 161–177.
Kumar, A., Prakash, A., Dogra, S., 2011. Protective effect of curcumin (Curcuma longa) against D-galactose-induced senescence in mice. J. Asian Nat. Prod. Res. 13, 42–55.
Lan, Z., Liu, J., Chen, L., Fu, Q., Luo, J., Qu, R., Kong, L., Ma, S., 2012. Danggui-Shaoyao-San ameliorates cognition deficits and attenuates oxidative stress-related neuronal apoptosis in D-galactose-induced senescent mice. J. Ethnopharmacol. 141, 386–395.
Lemos, L.N., Fulthorpe, R.R., Triplett, E.W., Roesch, L.F., 2011. Rethinking microbial diversity analysis in the high throughput sequencing era. J. Microbiol. Methods 86, 42–51.
Liu, H., Pei, X., Wang, J., Zhou, Y., Wang, L., Qi, B., 2018. Effect of loach paste on the liver and immune organs of D-galactose-induced ageing mice. Food Agric. Immunol. 29, 316–331.
Lu, J., Zheng, Y.L., Wu, D.M., Luo, L., Sun, D.X., Shan, Q., 2007. Ursolic acid ameliorates cognition deficits and attenuates oxidative damage in the brain of senescent mice induced by D-galactose. Biochem. Pharmacol. 74, 1078–1090.
Lykkesfeldt, J., Hagen, T.M., Vinarsky, V., Ames, B.N., 1998. Age-associated decline in ascorbic acid concentration, recycling, and biosynthesis in rat hepatocytes-reversal with (R)-α-lipoic acid supplementation. Faseb. J. 12, 1183–1189.
Monente, C., Bravo, J., Vitas, A.I., Arbillaga, L., Peña, M.P.D., Cid, C., 2015. Coffee and spent coffee extracts protect against cell mutagens and inhibit growth of food-borne pathogen microorganisms. J. Funct. Foods 12, 365–374.
Na, S., Chen, T., Meigui, H., Ping, L., Karangwa, E., Xiaoming, Z., Shuqin, X., Chengsheng, J., 2014. Transglutaminase cross-linking effect on sensory characteristics and antioxidant activities of Maillard reaction products from soybean protein hydrolysates. Food Chem. 136, 144–151.
Naughton, V., Mcsorley, E., Naughton, P.J., 2011. Changes in calcium status in aged rats fed Lactobacillus GG and Bifidobacterium lactis and oligofructose-enriched inulin. Appl. Physiol. Nutr. Metabol. 36, 161–165.
Nguyen, V.C., 2006. Toxicity of the AGEs generated from the Maillard reaction: on the relationship of food-AGEs and biological-AGEs. Mol. Nutr. Food Res. 50, 1140–1149.
Ogasawara, M., Katsumata, T., Egi, M., 2006. Taste properties of Maillard-reaction products prepared from 1000 to 5000 Da peptide. Food Chem. 99, 600–604.
Pan, L., Zhang, X., Wang, J., Ma, X., Zhou, M., Huang, L.K., Nie, G., Wang, P., Yang, Z., Li, J., 2016. Transcriptional profiles of drought-related genes in modulating metabolic processes and antioxidant defenses inlolium multiflorum. Front. Plant Sci. 7, 519.
Patrignani, M., Rinaldi, G.J., Lupano, C.E., 2016. *In vivo* effects of Maillard reaction products derived from biscuits. Food Chem. 196, 204–210.
Rufián-Henares, J.A., Morales, F.J., 2007. Functional properties of melanoidins: *In vitro* antioxidant, antimicrobial and antihypertensive activities. Food Res. Int. 40, 995–1002.
Sadigh-Eteghad, S., Majdi, A., McCann, S.K., Mahmoudi, J., Vafaee, M.S., Macleod, M.R., 2017. D-galactose-induced brain ageing model: a systematic review and meta-analysis on cognitive outcomes and oxidative stress indices. PLoS One 12, e0184122.
Saulnier, D.M.A., Molenaar, D., Vos, W.M.D., Gibson, G.R., Kolida, S., 2007. Identification of prebiotic fructooligosaccharide metabolism in Lactobacillus plantarum WCFS1 through microarrays. Appl. Environ. Microbiol. 73, 1753–1765.
Shen, Y.X., Xu, S.Y., Wei, W., Sun, X.X., Yang, J., Liu, L.H., Dong, C., 2002. Melatonin reduces memory changes and neural oxidative damage in mice treated with D-galactose. J. Pineal Res. 32, 173–178.
Sirivarasai, J., Kaojarern, S., Chanprasertyothin, S., Panpunuan, P., Petchpoung, K., Tatsaneeyapant, A., Yoovathaworn, K., Sura, T., Kaojarern, S., Sritara, P., 2015. Environmental lead exposure, catalase gene, and markers of antioxidant and oxidative stress relation to hypertension: an analysis based on the EGAT study. BioMed Res. Int. 2015, 856319.
Somoza, V., 2005. Five years of research on health risks and benefits of Maillard reaction products: an update. Mol. Nutr. Food Res. 49, 663–672.
Sönmez, M., Türk, G., Yüce, A., 2005. The effect of ascorbic acid supplementation on sperm quality, lipid peroxidation and testosterone levels of male Wistar rats. Theriogenology 63, 2063–2072.
Stadtman, E.R., 2006. Protein oxidation and aging. Science 257, 1220–1224.
Strober, W., 2010. Inside the microbial and immune labyrinth: gut microbes: friends or fiends? Nat. Med. 16, 1195–1197.
Sun, S.W., Yu, H.Q., Zhang, H., Zheng, Y.L., Wang, J.J., Luo, L., 2007. Quercetin attenuates spontaneous behavior and spatial memory impairment in D-galactose–-treated mice by increasing brain antioxidant capacity. Nutr. Res. 27, 169–175.
Tropek, R., Cerna, I., Straka, J., Kocarek, P., Malenovsky, I., Tichanek, F., Sebek, P., 2016. In search for a compromise between biodiversity conservation and human health protection in restoration of fly ash deposits: effect of anti-dust treatments on five groups of arthropods. Environ. Sci. Pollut. Res. 23, 13653–13660.
Tuohy, K.M., Hinton, D.J., Davies, S.J., Crabbe, M.J., Gibson, G.R., Ames, J.M., 2006. Metabolism of Maillard reaction products by the human gut microbiota–implications for health. Mol. Nutr. Food Res. 50, 847–857.
Valls-Bellés, V., Torres, M.C., Muñiz, P., Boix, L., González-Sanjose, M.L., Codoñer-Franch, P., 2004. The protective effect of melanoidins in adriamicyn-induced oxidative stress in rat isolated hepatocytes. J. Sci. Food Agric. 84, 1701–1707.
Woodmansey, E.J., 2007. Intestinal bacteria and ageing. J. Appl. Microbiol. 102, 1178–1186.
Wu, F., Lv, P., Yang, N., Jin, Y., Jin, Z., Xu, X., 2018. Preparation of Maillard reaction flavor additive from germinated wheat and its effect on bread quality. Cereal Chem. 95, 98–108.
Wu, S., Hu, J., Wei, L., Du, Y., Shi, X., Zhang, L., 2014. Antioxidant and antimicrobial activity of Maillard reaction products from xylan with chitosan/chitooligomer/glucosamine hydrochloride/taurine model systems. Food Chem. 148, 196–203.
Xia, T., Yao, J., Zhang, J., Zheng, Y., Song, J., Wang, M., 2017. Protective effects of Shanxi aged vinegar against hydrogen peroxide-induced oxidative damage in LO2 cells through Nrf2-mediated antioxidant responses. RSC Adv. 7, 17377–17386.
Yeh, S.L., Wu, T.C., Chan, S.T., Hong, M.J., Chen, H.L., 2014. Fructo-oligosaccharide attenuates the production of pro-inflammatory cytokines and the activation of JNK/Jun pathway in the lungs of D-galactose-treated Balb/cJ mice. Eur. J. Nutr. 53, 449–456.
Yu, M., He, S., Tang, M., Zhang, Z., Zhu, Y., Sun, H., 2018. Antioxidant activity and sensory characteristics of Maillard reaction products derived from different peptide fractions of soybean meal hydrolysate. Food Chem. 243, 249–257.
Zackular, J.P., Rogers, M.A.M., Iv, M.T.R., Schloss, P.D., 2014. The human gut microbiome as a screening tool for colorectal cancer. Cancer Prev. Res. 7, 1112–1121.