The anti-lipidemic role of soluble dietary fiber extract from okara after fermentation and dynamic high-pressure microfluidization treatment to Kunming mice

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Revised: 17 January 2020 / Accepted: 23 April 2020 / Published online: 28 April 2020
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Abstract The present study was conducted to evaluate the anti-hyperlipidemia ability of the dietary fiber extracted from okara in mice fed a high cholesterol diet. The dietary fiber was extracted from okara by combining fermentation with dynamic high-pressure microfluidization. An animal model was established to test the hypothesis that soluble dietary fiber, insoluble dietary fiber and total dietary fiber inhibit the fatty liver could be related to the total lipids and cholesterol including total cholesterol, triglyceride, low-density lipoprotein cholesterol and high-density lipoprotein cholesterol in the serum. Compared with mice fed with simvastatin, mice fed dietary fiber can significantly reduce their serum total cholesterol, low-density lipoprotein cholesterol, triglyceride, and atherogenic index whereas no significant effect on high-density lipoprotein cholesterol was observed. Dietary fiber lowered a high level of liver total cholesterol and triglyceride. The dietary fiber extracted from okara might play an important role in the prevention of hyperlipidemia in high cholesterol mice and could be used as a natural supplement to a high cholesterol diet of functional food, due to the suppression of liver lipid synthesis.

Keywords Hyperlipidemia · Soluble dietary fiber · Liver lipid · Anti-lipemic function · Okara

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Introduction

Hyperlipidemia is considered to be an important risk factor for developing cardiovascular disease, which has become a global health issue. A recommendation indicated that levels of total serum cholesterol (TC) could be raised by saturated fats, thereby putatively it also can increase the risk of atherosclerotic coronary heart disease (CHD) (DiNicolantonio et al. 2015). To treat hyperlipidemia, extensive interventions have been made, such as diet control, exercise, and hypolipidemic drugs. (Stone 1996). Although pharmaceutical interventions have proved to be effective against urgent occasions, many hypolipidemic drugs are known to induce adverse side effects (Howard & Udenigwe 2013). Emerging researches have focused on the application of natural food-based strategies in disease management to strengthen a strong positive link, which is between functional food components and human health (Howard and Udenigwe 2013). According to reports, several pharmaceutical plants in China have hypolipidemic effects, of which *Tephrosia Pers.* has a promising prospect of application. What's more, a present study concluded that due to the strong phenolic contents and radical scavenging activity, zedoary herbal tea played a protective role in anti-hypercholesterolemia and lipidemic conditions (Tariq et al. 2016). Similarly, after 5–10% of fresh mulberry (*Morus alba* L.) fruit in a 4-week diet treatment significantly had hypolipidemic and antioxidant effects in normal and hyperlipidemia (Yang et al. 2010). Its dietary fiber, fatty acids, phenolics, flavonoids, anthocyanins, vitamins and trace elements content may can explain these fresh mulberries (*Morus alba* L.) fruit benefits. Therefore, it can be speculated that dietary fiber might play an important role in reducing blood lipid.

Okara is the major surplus material of soybean products, and it has been usually considered as waste (Li et al. 2013). 1 kg of soybeans processed into soymilk or tofu can generate approximately 1.1 kg of fresh okara, and most of the okara isn't edible and is used for stock feed and fertilizer or dumped into the landfill (Khare et al. 1995). In addition, the characteristics of okara that has a high moisture content (70–80%) make it uneasy to manage and expensive to dry by traditional methods. However, there is a lot of dietary fiber that okara contains, which is healthy for human body, for it can lower blood fat and blood pressure, reduce the level of cholesterol in the blood, offer protection against coronary heart disease, and stop the appearance of constipation and colon cancer (Li et al. 2013). So many science researchers are looking at extraction dietary fiber from okara, hoping to turn the waste into treasure. Fortunately, some researches obtain new advances. A relevant experiment also showed more soluble dietary fiber (SDF) can be provided by okara by combining fermentation with DHPM (Tu et al. 2014).

Even though some biological effects of dietary fiber extracted from okara have been studied, few experimental evidence has demonstrated convincingly its lipid-lowering effects. In addition, a question in which chemical compounds in dietary fiber have potential in anti-hypolipidemic arises. As is known to all that SDF is rich in okara (Lin et al. 2020a, b). Based on this, it can be guessed that the chemical compositions of SDF are possibly crucial for lipid-lowering. Therefore, the present study was carried out to demonstrate and account for the anti-hyperlipidaemic activity of dietary fiber extracted from okara in mice after feeding on a high cholesterol diet, which combined fermentation with DHPM in the extraction of dietary fiber from okara. Furthermore, okara' extraction and chemical compositions of dietary fiber were also probed.

Materials and methods

Extraction of dietary fiber from okara

Fresh okara (tofu making) were kindly provided by a soy foods processor (Food Technology Institute, Nanjing Liuwei Food. Co. LTD. China), and was rinsed under running water several times to remove the proteins and other impurities until the filtrate was clean. The residue was dried and smashed for further research. *Aspergillus niger* (CICC 2238) and *Neurospora crassa* (CICC 40203) were obtained from the China Center of Industrial Culture Collection (China-CICC). The strains were cultured in de Man, Rogosa and Sharp (MRS) broth and cultivated at 28 °C for 24–48 h; each activated culture

was inoculated into 10 mL MRS broth and then cultivated at 28 °C for 24 h. The culture (540 nm optical density) was diluted by 0.85% sterile saline to get a solution containing 10^6 and 10^7 cfu/mL, and used as an inoculant for okara fermentation. In the experiment, we heated okara at 105 °C for 15 min, cooled 100 mL of okara concentrates (dry matter proportion between 8 and 10%) to the fermentation temperature (28 °C) and then inoculated it with 0.2% mixed culture consisting of equal proportions of both strains. The fermentation takes about 20 h and the ultimate pH of the fermentation product is 4.0 ± 0.3 . The product was lyophilized for further study.

The okara and the fermentation products were uniformly dispersed to a solid to liquid ratio of 1:30. Dynamic high-pressure microfluidization (DHPM) experiments were employed on laboratory scale devices (M-110EH, Microfluidics, Newton, USA). Treat the residue suspension at room temperature and under the pressure of 0, 140 and 180 MPa, respectively.

Fiber Official methods of analysis were employed to determine the related parameters in okara samples, such as moisture, protein, fat, total dietary fiber ash and calorie value (method 991.43, AOAC 1995). The hypothesis in this way is that the conditions we adopted to determine the dietary fiber content were similar to those discoveries in the human alimentary tract of the following enzymes: amyloglucosidase (A-9913, Sigma-Aldrich Co. LLC, St. Louis, USA) (60 °C, pH 4.0–4.7, 30 min), protease (P-3910, Sigma-Aldrich Co. LLC, St. Louis, USA) (60 °C, pH 8.2, 30 min) and heat-resistant α -amylase (A-3306, Sigma-Aldrich Co. LLC, St. Louis, USA) (100 °C, pH 8.2, 15 min). The insoluble dietary fiber (IDF) was filtered and then the residue was cleaned twice with 10 mL 70 °C distilled water. Combine the filtrate and the water used for washing, four bulks of 60 °C 95% ethanol was mixed to precipitate SDF. The filter was washed twice with 15 mL of 78% ethanol, twice with 15 mL of 95% ethanol, and twice with 15 mL of acetone, then dried it at 105 °C overnight and weighed. The blank sample was assessed in parallel to the real sample for the purpose of whether there was any possible contribution from reagents to the residue (Russo et al. 2014). The sum of IDF and SDF was total dietary fiber (TDF). Determination of neutral detergent fiber (NDF) and acid detergent fiber (ADF) according to Van Soest (1963), Soest & Van Wine (1967). Hemicellulose content originated in the difference between NDF and ADF contents. Determination of lignin content in ADF by Van Soest method (1963), which delimited it to be the insoluble lignin part in 72% H_2SO_4 . All data were expressed on a dry weight basis. The SDF was collected and stored at 4 °C before analysis of its chemical constituents by Gas Chromatography-Mass Spectrometry (GC-MS).

Determination of the composition of the dietary fiber after hydrolysis of trifluoroacetic acid. The sample was hydrolyzed in a sealed tube with 2 M trifluoroacetic acid at 100 °C for 6 h. Excess trifluoroacetic acid was evaporated under reduced pressure. The residue was continuously dissolved in methanol and steamed to dryness three times. The residue was redissolved in 2 mL of methanol then shifted to a glass tube. The solution was treated with nitrogen till dryness. Pyridine (0.5 mL) and hydroxylamine hydrochloride (10 mg) were added into the tube. The sealed tube was permeated in a constant temperature water bath at 90 °C for 30 min. Subsequently, 0.5 mL of acetic anhydride was added to the tube. The mixture was stored at 90 °C for 30 min (Rostami and Gharibzadeh 2016). The acid hydrolysis released the various fiber compositions: neutral sugars and uronic acid. Neutral sugars were determined by GC as acetate alditol. Glucose, mannose, galactose and arabinose were weighed 10 mg each, and then were placed in 2 mL reagent bottle where hydroxylamine hydrochloride 10 mg and 0.5 mL pyridine were added. The mixtures were Oscillated at 90 °C water baths and kept 30 min and cooled to room temperature. After adding 0.5 mL of acetic anhydride, the bottle was oscillated in 90 °C water baths and kept 30 min again. Acrylic esters of sugar derivatives were got which was diluted to 1:100 with methanol before being injected into the GC–MS system.

The SDF was diluted to 1:100 in methanol before being injected into the GC–MS system. The Gas Chromatography–Mass Spectrometry analysis was performed with the help of an Agilent 6890 instrument. The column was a DB-1701 fused silica capillary column 30 m (0.25 mm i.d., 0.25 µm film thickness). The oven temperature programming was set as follows: the ascending speed of 50–140 °C, 140–160 °C, 160–280 °C was 20 °C/min, 1 °C/min, 10 °C/min, respectively, and at 280 °C remained the same for 5 min. Temperatures of injector and detector were both 280 °C. The volume of the injector was 1 µL; the split ratio was 20:1, and the carrier gas was nitrogen. Identification depended on sample retention time data and comparison with authentic standards, electron impact-mass spectra (EI-MS) data and computer matching using NIST library (NIST = NIST. Mass Spectral Library, the National Institute of Standards and Technology, U.K, 1998). Figure 1 shows GC chromatogram of the SDF extracted from soybean meals.

Animal preparation

Male Kunming Mice weighing between 20 ± 2 g purchased from the Beijing Vital River Laboratory Animal Technology Co., Ltd., China, were used in the present

Fig. 1 The effects of dietary fiber on the serum TC, TG, HDL-C, LDL-C and AI in mice. NG: normal control mice fed with normal diet for 5 weeks. HCG: hypercholesterolaemic mice fed with high cholesterol (HC) diet for 5 weeks. TDFG: hypercholesterolaemic mice fed with HC diet for 5 weeks. During the last 3 weeks, TDF was daily administered by intragastric intubation. SDFG: hypercholesterolaemic mice fed with HC diet for 5 weeks. During the last 3 weeks, SDF was daily administered by intragastric intubation. IDFG: hypercholesterolaemic mice fed with HC diet for 5 weeks. During the last 3 weeks, IDF was daily administered by intragastric intubation. CG: hypercholesterolaemic mice fed with HC diet for 5 weeks. During the last 3 weeks, the reference drug simvastatin was administered daily at a dose of 40 mg/kg body weight (bw)

experiment. Although childhood or adolescent person's metabolism higher than adults or the elderly, the development of complications of hyperlipidemia require a longer period of time. Accordingly, most childhood or adolescent person do not feel the adverse reactions of hyperlipidemia. Therefore, a high cholesterol diet is fed to young mice to mimic the effects on young people. Mice were housed in a 12-h light–dark cycle chamber at a controlled temperature of 25 ± 2 °C and fed ad libitum with normal mouse food and tap water. Hypercholesterolemia was induced by the addition of 2.5 g of cholesterol powder in the normal mouse diet.

Six groups of ten or nine mice were established as followings:

NG: normal control mice fed with normal diet for five weeks. HCG: hypercholesterolemia mice fed with a high cholesterol diet for 5 weeks. TDFG: hypercholesterolemia mice fed with a high cholesterol diet for 5 weeks. TDF was performed daily through intragastric intubation during the last 3 weeks. SDFG: hypercholesterolemia mice fed with a high cholesterol diet for 5 weeks. During the last 3 weeks, SDF was daily administered by intragastric intubation. IDFG: hypercholesterolemia mice fed with a high cholesterol diet for 5 weeks. During the last 3 weeks, IDF was daily administered by intragastric intubation. CG: hypercholesterolemia mice fed with a high cholesterol diet for 5 weeks. At a daily dose of 40 mg / kg body weight (bw) of

the reference drug is administered simvastatin during the last 3 weeks. According to our preliminary research, low doses of simvastatin have little or no effect on reducing high serum cholesterol in high cholesterol mice. Increasing the dose of simvastatin to 40 mg/kg body weight resulted in a 35–40% reduction in serum cholesterol in high cholesterol mice.

In order to assess basal plasma cholesterol and triglyceride (TG) in all groups prior to dietary fiber or simvastatin administration functions, collecting fasting blood from the eyeball of the mice without anesthesia in the fifth week. The simplified experimental procedure as shown below.

The percent yield of TDF was 1.82 mL/100 g of fresh soybean meals which was about 1.82 mL/15.13 g dried soybean meals powder. The average of dried soybean meal powder consumption by the mice was 4.65 g/kg bw/day. So the daily dose of SDF, TDF and IDF administered in this research were calculated based on the above data (about 80 μ L/kg bw/day). SDF, TDF, and IDF were dissolved in a liquid paraffin emulsion at a concentration of 30 μ L/mL. 0.8 mL of liquid paraffin emulsion was administered to NG and HCG daily for the last 3 weeks. In order to improve the absorption of SDF, TDF, and IDF, the food was taken out for 2 h before administration.

The serum was separated from the blood with a centrifuge under the speed of 6000 rpm at 4 °C for 20 min (Brucklacher et al. 2008). The serum was preserved at – 20 °C preparing for the determination of the indicators. Blood was collected from the eyeball to determine the serum lipid profile, involving low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), TG and total cholesterol (TC). The atherogenic index (AI) is the ratio of TC-(HDL-C)/ HDL-C. The liver was extracted by the modified method of Folch and Lees (1957).

$$\text{RF}(\%) = \frac{W_1 - W_0}{S} \times 100$$

In formula: RF (Raw Fat)—liver fat content (%); W_1 —extraction filter paper packets before weight (g); W_0 —extraction filter paper packets after weight (g); S—liver samples dry weight (g).

Biochemical assay

Serum TC, TG, and HDL-C were assayed using an enzymatic kit (Catalog No. 120120, Gesellschaft für Biochemical und Diagnostica GmbH, Germany). LDL-C was calculated under the equation of LDL-C (m mol/L) = TC – (TG $\times$ 0.456 + HDL-C) (Fielding and Fielding 1982). TC and TG in the liver were determined using an enzymatic kit. A formula AI = (TC – HDL-C) / HDL-C calculation. The liver, heart, kidney, spleen are taken after

drawing blood from the animal and were dried with filter paper. The organ/body ratios were calculated (Cantalamesa and Nasuti 2003).

$$\text{Viscus ratios } (\%) = \frac{\text{viscus weight(g)}/\text{avoirdupois (g)}}{\times 100}.$$

RF Liver pathological assay

These mice were sacrificed and the liver tissue was removed. The largest in the liver of 5 mm from the edge of the smaller liver tissue was fixed with Bouins. The tissue was embedded with paraffin and was sliced, and then the tissue was colored with hematoxylin–eosin and observed by light microscopy.

Data and statistical analysis

All values are expressed as means $\pm$ SD. Statistical differences were calculated using a two-way analysis of variance (SPSS software version 19.5), followed by T test. Differences were considered significant at $P < 0.05$.

Results and discussion

Dietary fiber analysis

The influences of zymosis and DHPM on dietary fiber from okara are listed in Table 1. Okara' greatest fraction was dietary fiber (86.29 g/100 g), and IDF covered the main fiber fraction (75.91 g/100 g) (Mateos-Aparicio et al. 2008). The proportion of SDF to IDF in okara was about 0.137. The TDF content of okara was higher than previously reported by Redondo-Cuenca et al. (2008) (55.5 g/100 g) and Tu et al. (2014) (85.1 g/100 g). The proportion of SDF to IDF was almost the same as those findings in okara (Redondo-Cuenca et al. 2008; Tu et al. 2014). The differences could be due to growth or climatic conditions, processing methods or geographical sources.

DHPM treatment didn't remarkably affect TDF content but increased SDF and decreased IDF, both of which varied along with the changing DHPM pressure. The increase (10.38–25.93 g/100 g) in SDF became more obvious for DHPM-treated samples after fermentation, and the maximum increase was found in the sample at 180 MPa. In this case, a well-balanced proportion of IDF and SDF (IDF: SDF = 2.12) was obtained for okara. It was consistent with the results suggested by Tu et al (2014) that the proportion of SDF to IDF should range from 0.4 to 1.0.

Therefore, applying fermentation and DHPM treatment contributed to a redistribution from soluble to insoluble

Table 1 Effect of fermentation and DHPM on the content of dietary fiber and dietary fiber's fraction in fermented okara (g/100 g dry matter)

DHPM/(MPa)	0	140	180
UFTDF	79.35 ± 1.07 ^a	76.51 ± 1.32 ^a	73.77 ± 1.91 ^a
FTDF	86.29 ± 1.43 ^a	83.58 ± 1.75 ^a	80.95 ± 2.13 ^a
UFSDF	6.01 ± 0.37 ^a	14.13 ± 0.52 ^a	21.51 ± 0.91 ^c
FSDF	10.38 ± 0.54 ^a	23.67 ± 0.51 ^b	25.93 ± 1.43 ^b
UFIDF	75.91 ± 0.75 ^a	75.91 ± 0.75 ^a	75.91 ± 0.75 ^a
FIDF	75.91 ± 0.75 ^a	59.91 ± 2.32 ^b	55.02 ± 1.55 ^c
UFNDF	59.61 ± 1.21 ^a	53.38 ± 1.22 ^a	50.14 ± 1.03 ^a
FNDF	70.75 ± 1.56 ^a	58.97 ± 1.72 ^b	47.67 ± 0.55 ^c
UFADF(cellulose)	30.55 ± 1.01 ^b	30.01 ± 0.97 ^b	29.37 ± 0.99 ^b
FADF(cellulose)	39.77 ± 1.66 ^a	39.61 ± 0.73 ^{ab}	31.17 ± 0.57 ^b
UFADL	3.70 ± 0.42 ^a	3.34 ± 0.26 ^b	2.55 ± 0.11 ^a
FADL	9.73 ± 0.51 ^a	8.26 ± 0.43 ^b	6.45 ± 0.33 ^a
UF-hemicellulose	25.36 ± 0.91 ^a	20.03 ± 1.12 ^a	18.22 ± 1.05 ^a
F-hemicellulose	21.25 ± 0.23 ^a	11.10 ± 0.16 ^b	10.05 ± 0.63 ^b

DHPM dynamic high pressure microfluidization, FTDF fermented total dietary fiber, FIDF fermented insoluble dietary fiber, FSDF fermented soluble dietary fiber, FNDF fermented neutral detergent fiber, FADF fermented acid detergent fiber, FADL fermented acid detergent lignin, UFTDF unfermented total dietary fiber, UFIDF unfermented insoluble dietary fiber, UFSDF unfermented soluble dietary fiber, UFNDF unfermented neutral detergent fiber, UFAFDF unfermented acid detergent fiber, UFADL unfermented acid detergent lignin

Values are means ± SD, n = 3; means within a line with the different letters are significantly different ($P < 0.05$)

fiber. The reason why the TDF increased after fermentation was that the nutriment in okara, such as starch and protein, was metabolized by microorganisms. That microorganisms in the fermentation medium decompose the IDF may result in a general addition of SDF content after fermentation. The mechanism behind this was indistinct, but it probably related to an enzymatic or acid breakdown. Therefore, a previous study indicated that microorganisms were capable of hydrolyzing and metabolizing insoluble polysaccharides by generating extracellular enzymes (Schwarz 2001) and lactic acid.

The partial degradation of hemicellulose and cellulose to simple carbohydrates may decrease IDF content (Zia ur et al. 2003). High-speed impact, instantaneous pressure drop, high-frequency vibration, cavitation, strong shear and combined forces of ultra-high pressures belonging to DHPM may result in texture expansion and weak bond breakage between insoluble polysaccharides. Accordingly, IDF content decreased and SDF content increased obviously. While the pressure went from 150 to 200 Mpa, SDF polysaccharides may degrade into smaller fragments that

were soluble in ethanol and therefore had no conspicuous increase in SDF (Lambo et al. 2005).

To demonstrate whether lignin, hemicellulose or cellulose is partially degraded to soluble matter, we investigated the influence of fermentation and DHPM on dietary fiber content in okara (Table 1). Samples after fermentation contained 61.02 g/100 g NDF and 39.77 g/100 g ADF, respectively. Table 1 shows that lignin content of samples was rather little and cellulose content approximated ADF content. The content of cellulose at 140 MPa wasn't affected by the treatment of DHPM, but the treatment affected the hemicellulose content significantly. Treated samples at different pressures, the results showed that treatment at higher pressures had lower hemicellulose fiber content than samples at lower pressure. At 180 MPa after fermentation, hemicellulose content (10.05 g/100 g) was approximately half of that at 0 MPa (21.25 g/100 g). Hemicellulose was preferentially dissolved and dietary fiber was rich in cellulose. However, the cellulose treated with DHPM after fermentation had a slight reduction in cellulose. Therefore, we attempted to speculate that a mechanism that hemicellulose was degraded into some small molecular substances could account for the reduction of IDF. Furthermore, the looser and more porous fiber fabric that can be produced by fermentation made IDF degradation easier.

From the nutritional point of view, the redistribution of soluble to insoluble fiber in DHPM samples of okara may be useful (Mateos-Aparicio et al. 2010). For example, okara' health-promoting effects (Jiménez-Escrig et al. 2008), its fermentability in vitro and especially its bifidogenic capacity (Espinosa-Martos and Rupérez 2009), would benefit most under the situation that the soluble fraction could be significantly improved.

Therefore, the content of soluble fibers can be increased by choosing appropriate process conditions such as combining hydration, high hydrostatic pressure treatment, and temperature (Mateos-Aparicio et al. 2010). In addition, in the preparation of functional ingredients from okara for food fortification, high hydrostatic pressure treatment can be applied well and helpful (Mateos-Aparicio et al. 2010).

Monosaccharide components

Table 2 shows the monosaccharide composition of dietary fiber in fermented okara with DHPM. In general, SDF of okara consisted mainly of uronic acid and galactose, and contained less glucose, fucose, rhamnose, mannose, and xylose. Compared with SDF, IDF contained lesser mannose, rhamnose and fucose, and higher glucose, followed by galactose and uronic acid, as well as significant amounts of arabinose and xylose.

Table 2 Effect of DHPM on monosaccharides of dietary fibre in fermented okara (g/100 g dry matter)

DHPM/(MPa)	0	140	180
SDF	10.38 ± 0.54 ^a	23.67 ± 0.51 ^b	25.93 ± 1.43 ^b
Arabinose	1.63 ± 0.05 ^a	3.92 ± 0.15 ^{bc}	4.28 ± 0.21 ^c
Mannose	0.82 ± 0.03 ^a	0.56 ± 0.03 ^c	0.51 ± 0.02 ^b
Glucose	0.57 ± 0.02 ^{ab}	1.38 ± 0.06 ^c	1.27 ± 0.06 ^{bd}
Galactose	2.93 ± 0.09 ^a	7.89 ± 0.25 ^b	8.95 ± 0.29 ^b
Rhamnose	0.79 ± 0.03 ^a	1.71 ± 0.07 ^{ab}	1.12 ± 0.06 ^a
Fucose	0.35 ± 0.01 ^a	0.76 ± 0.03 ^b	0.69 ± 0.03 ^c
Xylose	0.21 ± 0.01 ^a	0.47 ± 0.02 ^c	0.19 ± 0.01 ^{ac}
Uronic acid	3.08 ± 0.11 ^a	6.98 ± 0.23 ^b	8.92 ± 0.30 ^b
IDF	75.91 ± 0.75 ^a	59.91 ± 2.32 ^b	55.02 ± 1.55 ^c
Arabinose	9.06 ± 0.31 ^a	4.25 ± 0.16 ^{bc}	3.02 ± 0.10 ^c
Mannose	3.62 ± 0.11 ^b	2.01 ± 0.09 ^b	1.93 ± 0.08 ^c
Glucose	24.18 ± 0.67 ^a	24.23 ± 0.63 ^c	25.01 ± 0.68 ^{bc}
Galactose	17.23 ± 0.68 ^a	12.13 ± 0.61 ^b	10.76 ± 0.58 ^{bc}
Rhamnose	2.25 ± 0.13 ^a	1.71 ± 0.12 ^b	1.22 ± 0.08 ^b
Fucose	2.05 ± 0.11 ^{ab}	1.72 ± 0.13 ^{ab}	1.51 ± 0.09 ^b
Xylose	4.58 ± 0.17 ^{ab}	2.21 ± 0.12 ^{ab}	2.01 ± 0.11 ^a
Uronic acid	12.94 ± 0.62 ^a	11.65 ± 0.59 ^{bc}	9.56 ± 0.53 ^b
DHPM/(MPa)	0	140	180
TDF	86.29 ± 1.43 ^a	83.58 ± 1.75 ^a	80.95 ± 2.13 ^a

DHPM dynamic high pressure microfluidization; TDF total dietary fibre; IDF insoluble dietary fibre; SDF soluble dietary fiber

Values are means ± SD, n = 3; Means within a column with the different letters are significantly different ($P < 0.05$)

The main reason for the increase in SDF in DHPM-treated samples was the increase of galactose, arabinose, glucose, fucose and uronic acid. In general, processing samples would use a higher pressure, SDF will have more uronic acid and neutral sugars content, this was due to the inactivation of pectinase including pectin polygalacturonase under high pressure. Moreover, under DHPM, fucose increased obviously, while rhamnose increased slightly in SDF (Table 2). As for IDF in DHPM-treated samples (Table 2), rhamnose, arabinose, xylose, fucose, galactose, mannose, and uronic acid were reduced. Meanwhile, samples treated with DHPM after fermentation had a slight increase in glucose (Table 2).

SDF content increased sharply due to the increase of arabinose, fucose, galactose, glucose, and uronic acid. It was noteworthy that concurrent decrease of arabinose, galactose, mannose, xylose, and uronic acid was reflected in IDF. DHPM was of less importance for other fiber monosaccharides which were the shortage of numbers. It can be speculated that hemicellulose and pectin which contains arabinose, galactose, and uronic acid were converted into SDF. Moreover, the decrease of mannose and

arabinose was sharp, while glucose and galactose increased slightly ($P \leq 0.05$) in most hydrated samples.

Serum TC, TG, HDL-C, LDL-C and AI

Serum TC of the high cholesterol-induced mice increased; serum TC of SDFG, TDFG, IDFG were already high fat, which was significantly lower than that of the NG (Fig. 1). The serum TG and TC had a similar trend. This experiment indicated that SDFG, TDFG, and IDFG had a significant function of lower serum TC and TG on hyperlipidemia mice. Through the conversion formula, we found that in the aspect of LDL-C and AI, SDFG, TDFG, IDFG were significantly lower than the HCG; IDFG higher than the SDFG, and TDFG and the CG were still significantly lower than the HCG. Atherosclerosis and the extent of AI were closely related. The results showed that the hyperlipidemia mice model was fed with the SDFG, TDFG and IDFG can increase HDL-C content but reduce TC, TG, LDL-C content and the AI value significantly. The reduction in serum TC was also found in animals fed diets enriched in dietary fiber from vegetable by-products as okara (Jiménez-Escrib et al. 2008; Villanueva et al. 2011). These results were demonstrated by several studies that assessed okara by-products as potential hypolipidemic ingredients and promotion for health properties in animals (Jiménez-Escrib et al. 2008; Villanueva et al. 2011). Therefore, SDFG, TDFG, IDFG had a significant role of improving lipid metabolism, reducing blood lipids, and preventing atherosclerosis.

Okara of dietary fiber on mice in the prevention of fatty liver

We found that the mice livers had little difference in size, but the surface of the livers from the HCG came out with some yellow spots, greasy in section. When it was dried and grinded, the livers of the HCG, TDFG and the IDFG appeared soft. While the livers of SDFG, NG, and CG were rather hard (Fig. 2). The high cholesterol diets were adopted in the experiment which can lead to serious fatty liver. Compare the fat contents of the livers in NG, TDFG, the IDFG, SDFG and CG with that of the HCG, NG were significantly lower. SDFG was significantly lower than IDFG and the CG, compared with HCG. Its inhibition rate of crude fat of the liver is 22.38%, the CG on liver fat content of the inhibition rate of 31.14%. The results showed that SDFG, TDFG and IDFG on the high cholesterol mice's fatty liver had a good curative effect.

Fig. 2 The content of RF in the liver and its inhibition rate of crude fat of the liver

Body weight ratio

Organic lipid accumulation and other diseases can be caused by long term high cholesterol diet. It can be known that the ratios of the liver, lung, heart, kidney to the bodyweight of the high cholesterol model group were higher than those of CG, SDFG, TDFG, and IDFG (Fig. 3). SDFG and TDFG spleen weight ratios were significantly higher than HCG, which spleen was an important immune organ to mice.

Mice liver pathology observation

Observation by eyes

The livers of the Kunming mice in NG had a normal size with smooth and sanguine. The size of the livers from the HCG increased with yellow and greasy appearance, which assumed a fatty liver.

Fig. 3 The ratio of viscous to body weight including liver, lung, kidney, heart and spleen

Light microscopy

HCG had congestion in the liver, and fatty degeneration was observed obviously from the liver cells (Fig. 4a). The liver cells were large with the round cell nucleus, liver leaflet rules, uniform cytoplasm, and the central vein to the cells around a bladder (Fig. 4c). Cells of TDFG (Fig. 4(d)) and the CG (Fig. 4b) were normal, fat liver cells occasionally varied, steatosis mildly, no necrotic liver cell and inflammatory cells, and the organization was close to normal. Within the framework of NG and IDFG, the number of fat drops of varying from the cavity, the nucleus was pushed to one side, moderate fatty degeneration (Fig. 4e). NG liver cells were neat and regular. Histopathology prosecution showed that a high cholesterol diet in long term can cause Kunming mice fatty liver. The SDFG, TDFG, and IDFG had a significant function which decreased the fatty liver. The liver of Kunming mice in each group will be the paraffin sections under the microscope in the optical observations.

Atherosclerosis which eventually caused coronary heart disease was a serious complication produced by hyperlipidemia. Nowadays, the number of hyperlipidemia patients has been continuously increasing, due to the unhealthy lifestyle, particularly a high-fat diet. Coronary heart disease morbidity can be decreased by diet regulation and drug therapy to command blood cholesterol subsequently (Austin et al. 1998). Though okara expressed the hypolipidemic function in common and diabetic animals, it was still unknown which compounds contribute to this effect. Since okara were rich in TDF, it was possible that TDF in okara was the key to the hypolipidemic action.

After a high cholesterol diet for 3 weeks, TC was obviously increased, and the level was still high in the next 2 weeks. High serum TC and LDL-C levels were reduced to levels comparable to those of CG during the last 2 weeks of SDFG, TDFG and IDFG treatment. Except for its anti-hypercholesterolemia effect, SDFG, TDFG also curbed a high serum TG level while no side effect was observed in high cholesterol mice compared to CG. The ability to lower the serum lipid profile and suppress a high level of AI suggested that SDF in okara in mice fed with a high cholesterol diet could be effective on the treatment of alleviating atherosclerosis in hyperlipidemia states. Various anti-hyperlipidemia mechanisms of SDF were proposed in the present study. The liver was a significant organ for lipid synthesis, it had a function on the serum lipid profile. The results of the experiment revealed that the SDF reduced TG and high liver cholesterol levels and had no remarkable function which was in okara (Villanueva et al. 2011). Some researchers had suggested that the cholesterol-lowering effect was related to the ability of the dietary fiber to promote the faecal neutral sterol and bile acid

Fig. 4 The Light microscopy of **a** the HCG, **b** the CG, **c** the SDFG, **d** the TDFG and **e** the IDFG

excretion, thus accelerating the transformation and decomposition of cholesterol to bile acids in the liver, and preventing the absorption of cholesterol from the circulation (Hong et al. 2007). It can be inferred that due to the suppression of liver lipid synthesis and the promotion of excretion and conversion of cholesterol, which made SDF have lipid-lowering action.

Lignin, cellulose, and hemicellulose can be combined with cholic acid so that high fat can be discharged directly from feces, thus consuming cholesterol in the body to supplement cholesterol consumed in bile, thus reducing blood lipid. Galactose and glucose, the monosaccharide compounds, are the main components of SDF extracted from okara (Table 2). According to some reports, galactose and glucose had a number of biological effects, such as

hypotensive and antioxidant effects (Mei et al. 2010). Moreover, it is showed that a high serum lipid profile in hyperlipidemia mice can be lowered by glucose (Sun et al. 2009). Therefore, glucose may be an important component of the lipid-lowering effect of SDF extracted from okara in mice fed with a high cholesterol diet. The deposition of cholesterol esters in arterial smooth muscle and macrophages can be boosted by vitro oxidation of LDL-C, which was associated with the evolvement of atherosclerosis (Steinberg 1995). Glucose has been discovered to have antioxidant effects and inhibits LDL-C oxidation, thus preventing atherosclerosis (Neri et al. 2010). High levels of serum lipids and AI in high cholesterol mice treated with okara in SDF may be attributed to galactose and glucose. Although SDF was involved in the anti-hyperlipidemia

effect of okara. However, the decline in serum lipid levels cannot be completely attributed to SDF. Therefore, the possible mechanisms for reducing lipid synthesis in the liver should be further clarified.

Conclusion

The results indicated that all of the TDF, SDF, and IDF possessed remarkable antilipidemic, the experimental high serum lipids were reduced in the TC, TG, LDL-C and AI. Moreover, with the SDF of TDF increasing, the anti-lipidemic activities increased. Compared to the group treated with a high cholesterol diet, TDF, SDF, and IDF can be analyzed for the decline in the ratio of the rat liver, lung, and heart, kidney to the body weight, proving that TDF, SDF, and IDF can mitigate the lipid accumulation. The spleen index of the SDF and TDF dose group increase significantly which might be related to promoting Kunming mice to improve immune function. The liver fat content of histopathology prosecution results showed that administering TDF, SDF and IDF can significantly reduce hyperlipidemia mice liver fat content, and reduce fatty degeneration of the liver cells, to maintain the normal shape. The participation of the reagents had a significant effect on the fatty liver. The effect of the SDF was the best, and the liver fat content reduced to 22.38%. Hyperlipidemia was often secondary to diabetes, kidney diseases caused by the diet. TDF, SDF, and IDF were non-toxic and non-side-effects, and they were effective in reducing blood lipids. It will have a broad prospect of developing healthy foods that can reduce blood lipids. It can be concluded that feeding the high cholesterol diet for 5 weeks increases serum lipids, AI, and liver lipids, while SDF treatment reduced hyperlipidemia and AI in high cholesterol mice over the past 2 weeks. Its anti-hyperlipidemia effect was mainly due to inhibition of liver lipid synthesis and the promotion of cholesterol excretion and transformation. Compared with the reference drug simvastatin, the anti-hyperlipidemia potency of the SDF extracted from okara was from its main monosaccharide compounds, especially galactose and glucose.

Acknowledgements The authors thank the National Natural Science Foundation of China (No. 41807364), the Sichuan Agricultural University 2019 College Students Innovation Training Program (No. 201910626137), the Education Department of Sichuan Province major project for financial support (No. 17ZB0338) and the Seedling Project of Sichuan Science and Technology Department (No. 2018125).

Compliance with ethical standards

Conflicts of Interest The authors declare that they have no competing interests.

Ethical approval Research ethical approval was waived by the China the Ministry of Health's National Biomedical Research Ethics Committee.

Informed consent Not applicable.

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