Enzymo-chemical preparation, physico-chemical characterization and hypolipidemic activity of granular corn bran dietary fibre

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Abstract Corn bran dietary fibre (CF) was paid more attention for its anticancer and hypolipidemic activities. In this paper, corn bran was firstly decomposed to the threadlike fibre (CF₁) by multiple enzymes and then further modified to the granular fibre (CF₂) by alkali under high pressure and high temperature (APT). The two types of fibres were characterized by scanning electron microscope (SEM) and near-infrared spectrophotometer (IR), and investigated by hydration measurements and nitrite adsorption assays. The results showed that CF₂ had more much specific surface area, and displayed 4.7, 6.3 and 30-fold increases in water retention (WR), swelling capacity (SC) and nitrite absorption (NA), compared with CF₁, respectively. The rat feeding trials showed that the granular fibre could decrease total cholesterol (TC), triglyceride (TG) and low density lipoprotein-cholesterol (LDLC) by 41.4 %, 20.7 % and 56.5 %, respectively. These excellent physiological activities indicate that CF₂ will be a potentially available dietary ingredient in functional food industries, and meanwhile imply that the enzymochemical method is a desired strategy for CF processing.

Keywords Corn bran · Dietary fibre · Process · Functional ingredient

Introduction

Corn as one of major agricultural crops was widely used as daily food, animal feed and natural material for starch

production (Rose et al. 2010). But corn bran was usually removed from corn during processing and threw away as trash (Rasper 1979; Wang and Liu 2000), which dramatically reduces the economic value of corn. Because corn bran contains abundant fibre and can be processed into useful CF by enzymatic or other methods (Negro et al. 2003; Schieber et al. 2001). CF is composed of polysaccharides and oligosaccharides as well as derivatives of them, which cannot be digested into absorbable nutrition by human digestive enzymes in the upper alimentary tract, but are beneficial of health maintenance and disease prevention (Thebaudin et al. 1997; Devin and George 2010). For examples, CF could be employed in medical nutrition therapies for maintenance of gastrointestinal health (Hu et al. 2008), prevention of hyperlipidemia (Dreher 2001) and colon cancer (Gallagher 2000), and regulation of calcium bioavailability (Tungland and Meyer 2002) and immunological system (Nimbe et al. 2009), etc. These applications were in basis of its binding capacity to bile acids (Kritchevsky 1995), cholesterol (Bartsch et al. 1990), steroid hormones (Babio et al. 2010), mineral elements (Nair et al. 1987), pesticide residues (Hu et al. 2010) and nitrites (Cammack et al. 1999; Duncan et al. 1997), etc. Especially the adsorption to nitrites could decrease the risk of cancer for that nitrites could react with amines and amides, e.g. amino acids, in the stomach to form the N-nitroso group of carcinogenic compounds (Archer 1989), which acted as mutagens in early stages of cancers in stomach, esophagus and bladder (Lu et al. 1986; Joossens et al. 1996). However, these physiological activities greatly depended on the technologies by which CF were processed well (Femenia et al. 1997; Gallagher and Schneeman 2001; Hüttner et al. 2010). Therefore, corn bran must be processed by feasible strategies to modify its chemical compositions rightly, so as to generate the desired CF with highly physiological activities (Garau et al. 2007; Guillon and Champ 2000; Verma and Banerjee 2010).

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In order to get high active CF, here corn bran was firstly processed to CF₁ by multiple enzymes and then modified to CF₂ by APT method. And both of them were characterized by SEM, IR, WR, SC and NA. Also the physiological activities of them were inspected by the rat feeding trials.

Materials and methods

Preparation of CF₁ and CF₂

Dry CB was provided by Jilin Ethanol Company. Enzyme preparations were purchased from Wuxi Enzyme Company and the dosages of them employed in performances were referred to the Co. standards: amylase, 5.7 IU/g; lipase, 3 IU/g; protease, 5 IU/g; cellulase, 1.9 IU/g. All pH values of solutions in processes were adjusted by 1 mol/L hydrochloric acid or sodium hydroxide. The detail processes were described as follow. 200 g CB was crushed in a mill with 80 mesh screen, and then processed in 1,000 mL deionized water by amylase at pH 6.5 and 60 °C for 15 min. Subsequently lipase and protease were added into the process solution to treat the sample at pH 7.0 and 40 °C for 30 min. Whereafter the sample was filtered through four layers of gauze, washed twice with 300 mL distilled water and dried in a drying oven at 60 °C for 48 h. Further the sample was processed in 1,000 mL deionized water by cellulase at pH 6.0 and 50 °C for 90 min, and then filtered, washed and dried in the same way, to get the product CF₁.

50 g CF₁ was further mixed with a 500 mL sodium hydroxide solution (0.1, 0.3, 0.6, 1, 3, 5, 7 or 10 %) and treated in a sterilization pot at 105 kpa and 121 °C for 30 min, and then filtered, washed and dried as the foresaid way. So CF₂ was obtained.

Characterization on CF₁ and CF₂

The procedures for structural characterization on CF₁ and CF₂ were same. First, 1 mg sample was suspended in 500 µL distilled water and then spread on a silicon slice (10×10×0.2 mm). After the slice was left in air for 12 h to let water volatilize, it was sputter-coated with gold for view the physiognomy on a scanning electron microscope (SEM, SSX-550, Shimadzu, Japan) with an accelerating voltage of 15 kV. On the other hand, 2 mg sample was weighed and mixed with 200 mg KBr by grinding in a mortar. The sample KBr wafer was made in a pressed powder machine and analyzed on an UV–vis–NIR spectrophotometer (IR, Shimadzu 3600).

Measurement of WR and SC

WR is defined as the mass ratio of fibre-binding water to dry fibre, which is showed by $WR \text{ (g/g)} = \frac{m_2 - m_1}{m_1}$. The m_1 is the mass of dry fibre. The m_2 is the mass of hydrated fibre, which

was gotten by mixing 1 g dry fibre with 75 mL water in a 100 mL centrifuge tube, and agitating the resulting suspension for 24 h and then centrifuging at 3,500 rpm for 30 min. The precipitate was the hydrated fibre. All samples were measured repeatedly ($n=3$).

SC is the ratio of the total volume of fibre after immersion and equilibration in excess water, to the original mass of dry sample, which can be written as $SC \text{ (mL/g)} = \frac{V}{m}$. The m is the mass of dry fibre, and the V is the final volume of fibre swelled with enough water, which can be obtained by putting 1 g dry fibre in a test tube with approximately 20 mL water for hydration about 24 h. The final volume of swelled fibre is V . All samples were measured repeatedly ($n=3$).

Adsorption to sodium nitrite

The assays for adsorption of sample CF₁ or CF₂ to nitrite were carried out by the following way. 0.5 g dried sample was

Fig. 1 Physiognomies of two fibres, CF₁ (a) and CF₂ (b), showed by scanning electron micrographs. CF₁: Threadlike corn bran dietary fibre prepared by processing corn bran with multiple enzymes; CF₂: Granular corn bran dietary fibre prepared by processing corn bran with multiple enzymes and with alkali under high pressure and high temperature, respectively

added to 50 mL sodium nitrite solution (2 mmol/L) in an Erlenmeyer flask. The mixture was adjusted to pH 2.5 by hydrochloric acid (1 mol/L) and then shaken at 37 °C for 24 h. During the course of shaking, 1 mL suspension was taken out for centrifugation after 5, 10, 15, 30, 40, 50, 60, 90, 120, 150, 180, 210, 240, 270 and 300 min, respectively. Every time, 500 μ L resultant supernatant was added in a 25 mL volumetric flask, in which two required reagents were added subsequently. They were 2.5 mL acetic acid solution (60 %, v/v) and 5 mL ingrain reagent (mixture of 1 g/L N-(1-Naphthyl) ethylenediamine dihydrochloride and 10 g/L sulfanilic acid, in equal volumes), respectively. In the end, enough distilled water was added in the flask to form a final 25 mL solution. The solution was incubated in dark for 25 min and the absorbance was reported at 550 nm on a SP-752 ultraviolet spectrophotometer. Sodium nitrite was quantified based on a standard curve constructed by the data of measurement on those concentration-various sodium nitrite solutions that lacked CF₁ and CF₂. All aforesaid assays were performed repeatedly ($n=3$).

Rat feeding trials

Forty-eight male Wistar rats were employed in feeding trials. Among them, 12 rats were randomly arranged as the control group (CG) fed with normal rat food (27 % corn flour, 19 % bran, 16 % rice, 16 % soybean, 13 % fish meal, 3 % calcium, 3 % bone meal, 2.3 % yeast powder, 0.5 % salt, 0.1 % compound vitamin and 0.1 % trace elements). Another 36 rats were constructed as the hyperlipidemia rat group (HG) fed with fat-high rat food (85 % normal rat food, 4 % cholesterol, 10 % lard, 0.3 % propylthiouracil and 0.7 % fish meal). After 28 days, the 36 rats were determined with high blood lipids

and further divided into three groups of 12 animals: (1) a model comparison group (MCG) that unceasingly received a diet with fat-high rat food; (2) a CF₂ group (CF₂G) that received a diet with 80 % fat-high rat food and 20 % CF₂; (3) a CF₁ group (CF₁G) that received a diet with 80 % fat-high rat food and 20 % CF₁. All rats were fed for 14 days and then 2 mL blood samples were taken from them for determination of TC, TG, LDLC and high density lipoprotein-cholesterol (HDL) on a biochemistry analyzer (BC-D800). The feeding trials were replicated three times and the results were analyzed by statistical software (SPSS).

Results and discussion

Process and microstructure

SEM showed that CF₁ contained many long threadlike fibres and that CF₂ was composed of abundant shape-irregular particles (Fig. 1). This implied that the multienzyme process was mainly to clear away mucilage in CB to form loose threadlike fibres in CF₁ and that the APT treatment was to further break these threadlike frameworks into granular fragments in CF₂. Namely, APT conditions had effectively broken some covalent bonds, e.g. glycosidic bonds, in CF₁ and thereby induced the threadlike fibres to collapse greatly into those amorphous particulates found in CF₂. The granular structures not only endowed CF₂ with more much specific surface area but also with more many hydroxyl groups. The increased hydroxyl groups can be affirmed by the IR spectra in which CF₂ has a sharply increased absorbance at around 3,340 cm^{-1} that meant hydroxyl group, relative to CF₁ (Fig. 2). It was just this

Fig. 2 Hydroxyl group absorptions of CF₂ (—) and CF₁ (---) at 3,340 cm^{-1} , showed by infrared spectra. CF₁: Threadlike corn bran dietary fibre prepared by decomposing corn bran with multiple enzymes; CF₂: Granular corn bran dietary fibre prepared by processing corn bran with multiple enzymes and with alkali under high pressure and high temperature (0.6 % NaOH, 105 kpa and 121 °C), respectively

Fig. 3 Swelling capacity (SC) and water retention (WR) values of CF₂ processed by alkali under high pressure and high temperature (APT). CF₂ sample 1–8: Granular corn bran dietary fibre prepared by processing corn bran with multiple enzymes and with alkali (0.1 %, 0.3 %, 0.6 %, 1.0 %, 3.0 %, 5.0 %, 7.0 % or 10 % NaOH) under high pressure and high temperature (105 kpa and 121 °C), respectively; The SC and WR values are mean $\pm$ SD of replicative experiments ($n=3$)

granular structures that help CF₂ to have enhanced hydration and adsorption to nitrite as follows.

Hydration and adsorption

As two important physiological parameters of dietary fibres, the WR and SC values of CF₁ were 4.3 % and 2.3 mL/g due to multienzyme process, respectively. Comparing with the singly enzymatic decomposition, the further chemical treatments by APT way under various concentrations of sodium hydroxide have heavily affected the hydration properties of CF₂, strongly increasing both WR and SC values. They increased with the

Fig. 4 Absorbance of ingrained nitrite solution after adsorbed by CF₁ (white square) or CF₂ (black square) for some time. CF₁: Threadlike corn bran dietary fibre prepared by decomposing corn bran with multiple enzymes; CF₂: Granular corn bran dietary fibre prepared by processing corn bran with multiple enzymes and with alkali under high pressure and high temperature (0.6 % NaOH, 105 kpa and 121 °C), respectively. All assays were performed repeatedly ($n=3$) and average values were taken

Table 1 Biochemical parameters of CG and HG fed for 28 days

Group No.	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
CG	2.01 $\pm$ 0.34	1.06 $\pm$ 0.29	0.71 $\pm$ 0.10	0.85 $\pm$ 0.22
HG	5.58 $\pm$ 2.04 **	0.66 $\pm$ 0.17 **	0.93 $\pm$ 0.15 **	4.19 $\pm$ 1.79 **

All values are means $\pm$ SD, $n=3$, ** $P<0.01$, very significantly different from CG. CG Control group; HG Hyperlipidemia rat group; TC Total cholesterol; TG Triglycerides; HDL-C High density lipoprotein-cholesterol; LDL-C Low density lipoprotein-cholesterol

increases in concentration of sodium hydroxide employed in APT treatments from 0.1 % to 3 %, and then decreased with the further increases in the basic concentration up to 10 %, but remained at least twice as high as when no sodium hydroxide was used (Fig. 3). In addition to good hydration properties, the yield of dietary fibre is also important for industrial-scale production, since it obviously affects profits. The yields of CF₂ were negatively correlated with the concentrations of sodium hydroxide employed in APT treatments, particularly at the concentrations between 0.1 and 1.0 %, suggesting that low concentrations promote greater yields. Therefore, we chose 0.6 % sodium hydroxide as the optimal concentration in terms of yield, and hence for potentially industrial production. This basic concentration resulted in that the WR and SC values of CF₂ were 4.7 and 6.3-fold greater than those of CF₁, respectively (Fig. 3), and left that the final waste solution had a pH no more than 8, meeting the commonly allowable effluent standard pH 6–9 (Dreher 2001).

The excellent adsorption capacity of CF to nitrites was another more important physiological characteristic required in functional food industries. It was usually analyzed by detecting the concentrations of remnant sodium nitrite in the tested solution in presence of CF with adsorption time prolonging. That is to termly report the absorptions of assay

Table 2 Biochemical parameters of CG, MCG, CF₂G and CF₁G differently fed for 14 days

No.	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
CG	1.92 $\pm$ 0.34	1.18 $\pm$ 0.34	0.68 $\pm$ 0.17	1.06 $\pm$ 0.17
MCG	13.20 $\pm$ 9.36	0.82 $\pm$ 0.14	2.46 $\pm$ 0.43	9.55 $\pm$ 7.80
CF ₂ G	7.73 $\pm$ 3.40 **	0.65 $\pm$ 0.20 *	2.39 $\pm$ 0.48	4.15 $\pm$ 2.58 **
CF ₁ G	13.87 $\pm$ 9.51	0.99 $\pm$ 0.68	2.34 $\pm$ 0.23	10.32 $\pm$ 9.09

All values are means $\pm$ SD, $n=3$, * $P<0.05$, significantly different from MCG. ** $P<0.01$, very significantly different from MCG. CG Control group; MCG model comparison group; CF₁G CF₁ group received a diet with 80 % fat-high rat food and 20 % CF₁; CF₂G CF₂ group received a diet with 80 % fat-high rat food and 20 % CF₂; HG Hyperlipidemia rat group; TC Total cholesterol; TG Triglycerides; HDL-C High density lipoprotein-cholesterol; LDL-C Low density lipoprotein-cholesterol

solution after treated with ingrain reagent, at 550 nm. Here the absorptions were proportional to the concentrations of sodium nitrite in solution. The results showed that both CF₁ and CF₂ had remarkable adsorptions to sodium nitrite, and especially in the initial stages they caused the concentration of sodium nitrite to decrease quickly (Fig. 4). Moreover CF₂ behaved an adsorption capacity obviously stronger than CF₁, for instance the characteristic absorbance of the tested system at 550 nm after 5 min being almost half as high in presence of CF₂ as in presence of CF₁. Though the absorption capacities of them had some decreases after 50 min, both of them still behaved obvious adsorptions to sodium nitrite. After 5 h, the adsorption capacity of CF₂ to sodium nitrite has achieved at 13.41 mg/g, being about 30 times the dosage of sodium nitrite adsorbed by CF₁. It was estimated that the average adsorption rate of CF₂ to sodium nitrite in assay solutions was at 24.96 mg/L·h, having a 1.7-fold increase relative to CF₁. According to the absorptivity, a 7–18 g daily intake of CF₂ could absorb 0.2–0.5 g toxic dose of nitrite to prevent human toxication. In addition, CF₂ was found to adsorb negligible amounts of glucose in vitro, implying that CF₂ has no potential effect on the glucose absorption in gastrointestinal tract. The aforesaid results showed that CF₂ was a more effective nitrite-adsorbed material relative to corn bran sold in market (Negro et al. 2003).

Rat feeding efficacy

In order to assess functions of CF₁ and CF₂ in reducing blood lipids of hyperlipidemia animals, 36 rats as HG were fed with a fat-high diet as described in *Rat feeding trials* section. After 28 days, these animals had blood TC, LDLC and HDLC higher than those 12 CG rats fed with normal diet (Table 1). After further feeding on hyperlipidemia rats with differing diets as described in *Rat feeding trials* for 14 days, it was found that the CF₂G rats exhibited those blood lipid indices lower than MCG (Table 2). The former had very significant decreases in TC and LDLC by 41.4 % and 56.5 % compared with the latter, respectively (Table 2). And the TG of CF₂G rats was also notably reduced by 20.7 % relative to MCG rats. Nevertheless the HDLC of CF₂G rats was decreased only by 2.8 % than MCG rats, which hinted that CF₂ had no disadvantageous impact on blood. As to CF₁G rats, no remarkable differences were found from MCG rats in terms of blood lipid indices. It was obvious that CF₂ was a more preferable functional component employed in diet therapy reducing blood lipids.

Conclusion

To summarize, APT treatment appears to be an effective method for glycosidic bond cleavage by which the long threadlike

fibres in CF₁ is transformed into the abundant granular textures in CF₂. As a result, CF₂ has more much specific surface area and more many hydroxyl groups, and can bind much water, nitrite and serum lipids, etc. These physiological activities suggest that CF₂ is a desired dietary constituent to reduce the risk of cancer by adsorbing dietary nitrites and prevent or possibly cure hyperlipidemia by lowering blood lipid concentrations.

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