ORIGINAL ARTICLE

The adsorption of lead(II) ions by dynamic high pressure micro-fluidization treated insoluble soybean dietary fiber

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Abstract Insoluble dietary fiber from soybean residue (SIDF) was treated with dynamic high-pressure microfluidization (DHPM) and used as adsorbent for Pb(II) ion. The effects of pressure on the Pb(II) adsorption capacity, primary cilia structure and surface topography of SIDF were determined using a gastrointestinal simulated model in vitro. SIDF (at pH 7.0) showed maximum binding capacity $(261.42 \pm 2.77 \text{ \mu} \text{mol/g})$, which was about 1.13 times higher than that of untreated sample (233.47 \pm 1.84 μ mol/g), when pressure reached 80 MPa. However, the net adsorption value of SIDF in a simulated small intestine $({\sim} 9 \text{ \mu mol/g})$ was significantly lower than that in the stomach (\sim 48 μ mol/g), because of the competitive adsorption of Pb^{2+} by pancreatin, cholate and several enzymes in the small intestine. In addition, the adsorption capacity of SIDF exhibited good linear relationship with the physicochemical properties of total negative charges, and the adsorption behavior presumably occurred on the surface area of granules fiber.

Keywords Dynamic high pressure microfluidization . Pb . Insoluble dietary fiber . Soybean residue

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Abbreviations

Introduction

Heavy metal pollution has caused greatest concerns because heavy metals tend to concentrate through the food chain and accumulate in living bodies, which cause serious problems for the environment, and human health. Among the heavy metals, Pb (II) is a ubiquitous and toxic agent (Goel et al. [2005](#page-7-0)). Pb^{2+} poisoning in humans can cause severe damage to kidneys, blood, blood-forming organs, central nervous system, and gastrointestinal tract; especially the child's physical and mental health (Lo et al. [1999;](#page-7-0) Goel et al. [2005\)](#page-7-0). Therefore, considerable researches have been conducted to reduce the concentration of Pb^{2+} in waste waters (Özer [2007\)](#page-7-0).

Dietary fiber is considered as a health-promoting food component that has shown great potential for binding heavy metal ions (Ou et al. [1999;](#page-7-0) Özer and Pirincci [2006;](#page-7-0) Daou and Zhang [2014](#page-6-0); Mehta et al. [2015](#page-7-0)). Consumption of dietary fiber could increase the faeces metal content, preventing the body from heavy metal's toxicity (Serguschenko et al. [2007\)](#page-7-0). Insoluble dietary fiber as a by-product is abundant, inexpensive, and has high dietary fiber content (O'Toole [1999;](#page-7-0) Ou et al. [1999;](#page-7-0) Daou and Zhang [2014\)](#page-6-0). Modified dietary fiber

using chemical, physical and enzymatic methods have been recently reported to enhance their heavy metal adsorption capacity (Feng et al. [2009](#page-7-0); Park et al. [2013](#page-7-0)). However, few studies report on the modifying SIDF to enhance its heavy metal adsorption capacity.

Dynamic high pressure microfluidization (DHPM) is an emerging homogenization technology, which uses the combination-forces of high-velocity impact, highfrequency vibration, cavitation and ultra-high pressure shear to process macromolecular materials such as proteins, lipids, and carbohydrates (Iordache and Jelen [2003;](#page-7-0) Huang et al. [2013](#page-7-0)). Few reports focus on the physical properties of soybean dietary fiber influenced by DHPM. Liu et al. ([2006\)](#page-7-0) had reported the effect of instantaneous high-pressure treatment (IHP) on rheological properties of soybean dietary fiber. In our previous research, the composition and physicochemical properties of dietary fiber from soybean residue changed after DHPM treatment (Tu et al. [2014](#page-7-0)). However, the effect of DHPM on SIDF, in particular the adsorption capacity of Pb (II), has never been reported previously.

This study aimed to investigate the effect of DHPM-treated SIDF on the adsorption behavior of Pb^{2+} and its digestibility in vitro, and to analyze the relationship between the physicochemical properties of modified SIDF and its Pb(II) adsorption capacity. Meanwhile, the adsorption mechanism of Pb^{2+} by SIDF was also discussed by measuring structural characteristics. The modified insoluble dietary fiber is anticipated to develop products in food industries to meet consumers' demands.

Materials and methods

SIDF preparation and DHPM treatment

Soybean residue was obtained from a local fast food restaurant (The Dongbei family, Nanchang, China). The fresh residue was dried at 55° C for 24 h in a forced oven, ground and sieved to obtain uniform particles sizes $(\sim 0.15 \text{ mm})$. Starch and protein were initially removed from the samples to prepare the SIDF. After centrifugation at 3000 g for 20 min, the supernatant was discarded and the residue was washed twice with distilled water (70 \degree C) and then by 95 % ethanol. The washed precipitate was sequentially freeze-dried.

The freeze-dried SIDF samples were dissolved in distilled water and stirred at room temperature for 10 min. The mixture was homogenized twice in a homogenizer (GYB1003, Shanghai Donghua manufacturer, China) at 30,000 psi. The mixture was then treated with DHPM (M-110EH Microfluidics, Newton, USA) for 2 passes under 40, 80, 120

and 160 MPa, which were referred as SIDF-40, SIDF-80, SIDF-120 and SIDF-160, respectively. Samples without DHPM treatment was used as control and named SIDF-0. All SIDF were sequentially freeze-dried and stored in a desiccator.

Adsorption process of SIDF for Pb^{2+} under stimulated gastrointestinal tract

The preparation of digestive fluids was as follows. The simulated saliva solution comparised 2.38 g of $Na₂HPO₄$, 0.19 g of $KH₂PO₄$, 8.0 g of NaCl and 0.0054 g of α-amylase, which were dissolved in 1 L of distilled water. The solution was adjusted to pH 6.75 with 2 M NaOH or HCl. The simulated gastric juice: contained 0.32 % of pepsin prepared in 0.03 M NaCl. The solution was adjusted to pH 1.2 with 2 M HCl. Simulated intestinal juice included 0.05 g of pancreatin and 0.3 g of bile extract, which were dissolved in 35 mL of 0.1 M NaHCO₃ (Gawlik-Dziki et al. [2009\)](#page-7-0).

Each sample was passed through a simulated in vitro digestion model that consisted of gastric and intestinal phases (Espinal-Ruiz et al. [2014](#page-6-0)). The procedure consists of two main steps, namely an adsorption process under the simulated mouth and gastric conditions for 30 min at 37 °C and an adsorption process under the simulated colonic conditions for 60 min at 37 $^{\circ}$ C (Zacherl et al. [2011](#page-7-0)).The model of a simulated mouth included 10 mL of simulated saliva solution, which was added to 0.1 g of SIDF in a 50 mL centrifuge tube with a cap. After incubating at 37 °C for 10 min at 120 rpm in a shaker, the pH of the solution was adjusted to 2.0 with 5 M HNO₃. Then 0.1 mL of $Pb(NO_3)$ ₂ (100 mg) Pb^{2+}/mL) and 10 mL of simulated gastric juices were added, and then incubated at 37 °C for about 20 min at 120 rpm. After incubation, counting was initiated, and 0.1 mL of supernatant (sample was performed 2 times) was collected to determine Pb(II) ions content at 0, 5, 10, 20 and 30 min. The model of gastric condition was prepared as follows: the pH of the solution was initially adjusted to 7.0 with 2 M NaOH. Afterwards, 10 mL of the simulated intestinal juice was placed into the tube and incubated at 37 °C in a shaker at 120 rpm. About 0.1 mL of supernatant $(x2)$ was collected and used to determine the Pb(II) ions content at 0, 5, 10, 20, 30 and 60 min. The blank group was used without SIDF.

For each sample, supernatant was separated from adsorbents by centrifugation at 4000 g for 10 min and diluted with double distilled water to suitable concentrations. An atomic absorption spectrophotometer was used to determine the Pb(II) ion concentration in supernatant. The total adsorption capacity of Pb(II)

ion adsorbed (mg/g) and the net adsorption capacity (mg/g) were calculated using the following equations:

$$
AC_{\text{total}} = \frac{(C_0 - C_t)V}{W}
$$

$$
AC_{\text{net}} = \frac{(C_t^b - C_t)V}{W}
$$

where C_0 is the initial concentration of Pb(II) ions in the solution (mg/L), C_t is the concentration of Pb(II) ions in solution at t minute of the experimental groups (mg/L), C_t^b is the concentration of Pb(II) ions in solution at t minute of the blank group (mg/L), V is the volume of the solution (L), and W is the weight of the adsorbent (g).

The BC_{max} and BC_{min}

The BC_{max} and the BC_{min} of SIDF for Pb(II) ions were determined according to the methods of Zhang et al. [\(2011](#page-7-0)). Briefly, SIDF (0.2 g) was suspended in 20 mL of solution containing 10 mM of $Pb(NO₃)₂$. The pH was adjusted to 2.0 and 7.0 to simulate the conditions in the stomach and small intestine, respectively. The mixture was shaken at 37 °C for 3 h at 120 rpm in a water-bath incubator. Then, the sample (2 mL) was collected and centrifuged at 4000 g for 20 min. The concentrations of Pb^{2+} in the supernatant were determined by an atomic absorption spectrophotometer. SIDF (0.5 g) and $Pb(NO₃)₂$ (500 μ M) were used to measure BC_{min} . BC_{max} was measured using the same processes as that of BC_{min} .

Water-holding capacity

The water-holding capacity (WHC) of SIDF was determined following the method of Chau and Huang [\(2003\)](#page-6-0). SIDF was mixed with distilled water $(1:10, w/v)$ for 24 h. After centrifugation at 3000 g for 15 min, the supernatant was discarded, the residue was weighed, and the WHC was calculated as grams of water held by 1 g of SIDF.

Oil-holding capacity

The oil-holding capacity (OHC) was measured under the same conditions as WHC measurement, but commercial peanut oil was used instead of water. OHC was expressed as grams of peanut oil held by 1 g of SIDF.

Cation exchange capacity (CEC)

The cation exchange capacity (CEC) was determined using the method of Jiménez et al. ([2000\)](#page-7-0). Samples (0.1 g of SIDF (×4)) were suspended in 10 mL of 2 M HCl, stirred continuously for 24 h, and centrifuged for 15 min at 2500 g. The residue was

Fig. 1 Total adsorption capacity of Pb^{2+} by SIDF under the simulated gastrointestinal model (contact time 1 to 5 represent 0, 5, 10, 20, 30 min in simulated stomach; contact time 6 to 11 represent 0, 5, 10, 20, 30, 60 min in simulated small intestine)(a); Net adsorption process of Pb^{2+} by different SIDF samples in the simulated stomach (b) and simulated small intestine (c). SIDF-0, SIDF-40, SIDF-80, SIDF-120 and SIDF-160 indicates the SIDF was treated by DHPM under 0, 40, 80, 120 and 160 MPa, respectively

Values are presented as mean \pm SD ($n = 3$). Values in the same column with different letters are significantly different according to Duncan's multiple range tests ($p < 0.05$). SIDF-0, SIDF-40, SIDF-80, SIDF-120 and SIDF-160 indicates the SIDF was treated by DHPM under 0, 40, 80, 120 and 160 MPa, respectively

washed with distilled water until the pH of the supernatant was above 4.0. The acidic residue was suspended in 10 mL of 0.3 M NaCl (experiment was performed atleast 3 times) together with a blank containing distilled water. The supernatant was then titrated with 0.01 M NaOH. The CEC was expressed as mM of $Na⁺$ per dry sample.

Total negative charge (TNC)

The total negative charge (TNC) was determined following the method of Marshall et al. [\(1999\)](#page-7-0). Samples (0.5 g of SIDF at pH below 3.0) were suspended in 25 mL of 0.1 M NaOH in a conical flask with stopper, shaken at 25 °C for 24 h and centrifuged for 10 min at 2650 g. Then, 10 mL of the supernatant was added to 15 mL of 0.1 M HCL. Finally, the mixture was titrated with 0.1 M NaOH. The TNC was expressed as mM of H^{\dagger} per gram dry sample.

Fourier transform infrared (FTIR) spectroscopy analysis

Spectra of the freeze-dried samples were obtained at a phase resolution of 4 cm−¹ and averaged at 64 scans/min. Spectra were recorded in the transmittance mode from 4000 cm−¹ to 400 cm−¹ using FT-IR (Nicolet 5700;Thermonicolet, USA).

Scanning electron microscopy analysis

The microstructure of the samples were determined using an environmental scanning electron microscope (Quanta200F, FEI Deutschland GmbH, Kassel, Germany) at 10 kV voltage and $1000 \times$ magnification.

Statistical analysis

Data was analyzed using the analysis of variance (ANOVA) and was expressed as mean values from three replicates with standard deviations. The differences between mean values were established using Duncan's multiple range tests at the level of $P \le 0.05$. The statistical analysis was performed by SPSS (version 19.0).

Results and discussion

Adsorption process of SIDF for Pb^{2+} under stimulated gastrointestinal tract

The Pb^{2+} adsorption process of SIDF under simulated gastrointestinal tract was shown in Fig. [1](#page-2-0). Figure [1](#page-2-0)a shows that the total adsorption capacity of Pb(II) ions for SIDF increased

Values represent the means of 3 replicates $(n = 3) \pm$ standard deviation. Values in the same column with different letters are significantly different $(p < 0.05)$. WHC water-holding capacity, OHC Oil-holding capacity, CEC cation exchange capacity, TNC total negative charge. SIDF-0, SIDF-40, SIDF-80, SIDF-120 and SIDF-160 indicates the SIDF was treated by DHPM under 0, 40, 80, 120 and 160 MPa, respectively

Table 3 Person's coefficient correlation (r) between the physicochemical properties and BC_{max}, BC_{min} of insoluble dietary fiber from soybean residue

	WHC	OHC	CEC	TNC
BC_{max}	0.639	-0.359	-0.174	0.923 [*]
BC_{\min}	-0.579	0.490	0.027	-0.951 [*]

 BC_{max} maximum binding capacity, BC_{min} minimum binding concentration, WHC water-holding capacity, OHC Oil-holding capacity, CEC cation exchange capacity, TNC total negative charge

* means significant correlation

rapidly during the first 5 min in the stomach. The AC_{total} values of neutral small intestine were higher than those of the acidic stomach conditions, which was agreed with the results of numerous studies (Hu et al. [2010;](#page-7-0) Ou et al. [1999](#page-7-0); Dubey and Shiwani [2012](#page-6-0)). However, the net adsorption (the adsorption capacity of samples deducting the blank value) exhibited an adverse trend. Comparing Fig[.1](#page-2-0)b with Fig[.1](#page-2-0)c, the net adsorption of modified SIDF in the acidic stomach $({\sim} 48 \text{ \mu}$ mol/g) was markedly higher than that in the simulated small intestine (\sim 9 μ mol/g). The adsorption value of unmodified SIDF in the small intestine $({\sim 87 \text{ \mu}mol/g})$ was much higher than that in the stomach (\sim 43 μ mol/g). The higher value may mainly be attributed to the in vitro model that consist of complex enzymatic system, chalet and inorganic ions. The results indicated that the simulated digestive juices exhibited stronger Pb^{2+} binding ability than SIDF at pH 7.0. The generated suspended particles may comprise complex compounds of Pb^{2+} and simulated digestive juices. The results also showed that both the total and net adsorption capacities of Pb^{2+} were the highest than others when DHPM treatment pressure reached to 160Mpa, indicating that DHPM could increase the adsorption of SIDF to some extent.

Fig. 2 Effect of DHPM pressure on FT-IR spectra of SIDF (A); the FTIR spectrum of untreated sample before and after adsorbing Pb^{2+} in aqueous solution and simulated digestive juices (B) (Adsorbed Pb^{2+} a SIDF-0 after adsorbing Pb^{2+} in aqueous solution; Adsorbed Pb^{2+} **b** SIDF-0 after adsorbing Pb^{2+} in simulated digestive juices). SIDF-0, SIDF-40, SIDF-80, SIDF-120 and SIDF-160 indicates the SIDF was treated by DHPM under 0, 40, 80, 120 and 160 MPa,

respectively

The BC_{max} and BC_{min}

The values of BC_{max} and BC_{min} of SIDF for Pb (at pH = 2.0) and $pH = 7.0$) are shown in Table [1](#page-3-0). The BC_{max} values were much higher at pH 7.0 than those at pH 2.0. This result may be due to the stronger electrostatic repulsion forces between the positively charged H_3O^+ and Pb²⁺ at acidic pH. Conversely, more speciation of the surface function groups including – COOH, −OH were exposed in the solution at pH 7.0, thereby, adsorbing more Pb^{2+} . Consistent with the observation by Ou et al. [\(1999](#page-7-0)), all SIDF exhibited low BC_{min} values, indicating high affinity in the tested small intestine. DHPM treatment could enhance the BC_{max} values and treated fibers possess higher affinity for Pb(II), probably due to the changed structure and composition of SIDF. The values of AC_{total} and BC were clearly in contrast, which may be due to the in vitro model that consisted of complex enzymatic system, chalet and inorganic ions. In our previous study, DHPM increased the content of soluble dietary fiber, reducing the hemicelluloses and damaging the structure of dietary fiber to form a rugged surface (Tu et al. [2014](#page-7-0)). However, no significant difference ($P < 0.05$) was observed between BC_{max} and BC_{min} at pH 2.0 as pressure increased. Based on the chemisorption mechanism, the adsorption effect of the exposed surface function groups may be concealed by H_3O^+ in the acidic solution while emerging from the neutral solution.

Physicochemical properties and their relationship with BC_{max} and BC_{min}

The effect of DHPM on the physicochemical properties including WHC, OHC, CEC and TNC of SIDF is shown in Table [2.](#page-3-0) Compared with control, DHPM could decrease the values of WHC and OHC, indicating that increasing pressure may change the structure of SIDF. SIDF-80 and SIDF-40

exhibited the highest values of WHC and OHC, respectively, implying that DHPM could expose the hydrophilic and hydrophobic groups of SIDF. SIDF exhibited the lowest values of WHC and OHC when pressure reached to 160 MPa, indicating that the SIDF structure was damaged. In addition, CEC increased as the pressure increased. This result was due to the high-frequency vibration, powerful shear and cavitation force effect of DHPM, which enhanced the content of –OH, −COOH, and -NH2 as pressure increased, thereby improving the CEC capacity. However, TNC initially increased when DHPM pressure increased from 0 MPa to 80 MPa, and then decreased as pressure increased to 120 MPa and 160 MPa. In

addition, Table [3](#page-4-0) shows that the BC_{max} and TNC gained a good linear relation. The BC_{min} values exhibited a negative correlation with TNC values, indicating that the maximum amount of Pb^{2+} adsorption and affinity of SIDF depended on the total negative charges of SIDF. However, the relationship among BC_{max} , BC_{min} , water holding ability and oil holding ability was very weak.

FT-IR analyses

FTIR spectra of SIDF unmodified/modified by DHPM under different pressure levels are shown in Fig. [2a](#page-4-0). The spectra

Fig. 3 Environmental scanning electron micrograph of SIDF a SIDF-0: b SIDF-40; c SIDF-80; d SIDF-120; e SIDF-160; f SIDF-0 after adsorbing Pb^{2+} in aqueous solution; g SIDF-0 after adsorbing Pb^{2+} in

simulated digestive juices. SIDF-0, SIDF-40, SIDF-80, SIDF-120 and SIDF-160 indicates the SIDF was treated by DHPM under 0, 40, 80, 120 and 160 MPa, respectively

were rather similar for all the samples. No significant difference in characteristic absorption bands was observed at 3411 cm⁻¹ (v(-OH)), 2926 cm⁻¹ (v(-CH)), 1750– 1500 cm⁻¹ ($v(C=O)$), and 1055 cm⁻¹ ($v(C-O-C)$). This result showed that DHPM treatment had no effect on the primary structure of SIDF, which was agreed with findings of Chen et al. (2012) on the primary structure of pectin. The chemical structure of SIDF-0 and the ones after Pb^{2+} adsorption in aqueous solution and simulated digestive juices, are illustrated in Fig[.2b](#page-4-0). Compared with the un-adsorbed Pb(II), the wavenumber around 3411 cm⁻¹ of SIDF with adsorbed Pb(II) (a and b) slightly shifted towards the left, indicating that the oxygen negative charge mainly adsorbed Pb^{2+} . Compared with the adsorbed Pb^{2+} in aqueous solution (a), the wavenumber of the adsorbed $Pb^{2+}/Pb(II)$ in simulated digestive juices (b) shifted left, demonstrating that SIDF exhibited higher $Pb^{2+}/Pb(II)$ adsorption capacity in simulated digestive juices.

Scanning microscopy analysis

The surface structure images of unmodified/modified SIDF, native SIDF after adsorption in $Pb(NO₃)₂$ solution (pH 7.0), and SIDF in simulated small intestine are presented in Fig. [3.](#page-5-0) As shown in Fig. [3a](#page-5-0), the unmodified SIDF exhibited a relatively tidy structure with massy fibrous. However, the thick pieces were sheared into many small and thin alveolate fragments upon treatment with DHPM at pressure 40 and 80 MPa (Fig. [3b](#page-5-0) and Fig. [3](#page-5-0)c). However, many amorphous, loose, and agglomerate pieces appeared (Fig. [3d](#page-5-0) and Fig. [3](#page-5-0)e), as treatment pressure increased to 120 MPa and 160 MPa. The appearance of agglomerates indicates that DHPM could induce degradation of dietary fiber from soybean residue because of its powerful shear force, high-velocity impact and highfrequency vibration. These results also indicated that the lacerate small pieces could be reunited by increasing the DHPM treatment intensity to a certain extent.

The structure of fiber matrix affected its adsorption and physicochemical properties (Sangnark and Noomhorm [2003\)](#page-7-0). The adsorption of Pb^{2+} by SIDF was dependent on the structure of fiber matrix. When pressure reached 40 to 80 MPa, the surface structure of SIDF changed from massy pieces to small and thin alveolate fragments that exposed more adsorption surface area, binding sites, and groups, resulting in increased BC_{max} values. However, when the fragments reunited into large pieces at 120 and 160 MPa, the surface area decreased relatively and the adsorption sites and groups were hidden, resulting in the decrease of adsorption capacity.

Figure [3](#page-5-0)f shows the image of SIDF-0 binding Pb^{2+} in the normal aqueous-solution-adsorption pattern. Compared with Fig. [3a](#page-5-0), Fig[.3](#page-5-0)f shows abundantly unfolded fiber bundles on the outer surface of SIDF. However, the surface seems to be completely unfolded for other small and thin particles.

Assuming that Pb^{2+} adsorption mainly occurred on the exposed part of particles including the pores, SIDF-40 and SIDF-80 are concluded to possess the highest adsorption capacity (Table [1](#page-3-0)). Figure [3g](#page-5-0) shows that the image of the generated complex chunk was completely different from that of the dietary fiber. The mixture may contain the complexes of small fiber fragments, cholate, pancreatin and some enzymes that entrapped large amounts of Pb(II) ions and other inorganic ions. Meanwhile, the abnormal phenomenon of the net adsorption capacity of Pb^{2+} by SIDF in the simulated small intestine can result from the Pb(II) ions adsorption capacity by pancreatin, cholate and other enzymes superior to that of SIDF.

Conclusions

DHPM could increase the Pb^{2+} adsorption by SIDF to some extent. DHPM treatment changed the surface structure of SIDF, but did not affect the primary structure. Adsorption may occur on the surface area of fiber granules, and adsorption ability depended on its structure and physicochemical property. The adsorption capacity (BC_{max}) and affinity exhibited a good linear relationship with the physicochemical property of total negative charges. Moreover, we found that the net absorption of SIDF in the simulated small intestine $(\sim 9 \mu m o l/g)$ was much lower than that in acidic stomach $(\sim 48 \text{ \mu mol/g})$ in the in vitro simulated gastrointestinal model. This result may be due to the competitive adsorption of Pb^{2+} by pancreatin, cholate, and other enzymes in the small intestine.

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