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Arabinoxylan from *Canna edulis* Ker by-product and its enzymatic activities

Juan Zhang[a,*], Zheng-Wu Wang[b,*]

[a] School of Life Sciences, Shanghai University, Shanghai 200444, PR China
[b] Department of Food Science and Technology, School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai 200240, PR China

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ABSTRACT

Arabinoxylan (AX) was extracted and purified from *Canna edulis* Ker by-product. Through column chromatography, AX was further separated, leading to the isolation of two single compounds, namely, AXI and AXII. Moreover, the structures of AXI and AXII were characterized by GC, GC–MS and NMR. The result indicated that arabinose and glucuronic acid occurred at 1,4-linked xylose units as backbone at positions 3 and 2 in both AXI and AXII with varying ratios, respectively. Furthermore, the effects of AX on enzymatic digestibility of β-lactoglobulin and tributyrin hydrolysis by lipase were evaluated. The results showed that AX had obvious inhibition effects on pepsin and lipase activities, and decreased β-lactoglobulin digestibility and tributyrin hydrolysis as well. It indicates that *C. edulis* AX could be used as a functional food ingredient.

1. Introduction

It has been reported that arabinoxylan (AX) has some interesting functional properties, such as viscosity enhancement, gel formation, possibly foam stabilization and prebiotic activity (Bergmans, van Dijk, Beldman, & Voragen, 1999; Courtin & Delcour, 2002; Grootaert et al., 2007; Hollmann & Lindhauer, 2005; Schooneveld-Bergmans, Van Dijk, Beldman, & Voragen, 1999). Moreover, it can absorb large amounts of water and influence significantly the water balance, rheological properties of dough, and the retrogradation of starch and bread quality. Many potential applications have been reported, including the use as a functional ingredient or soluble fiber source in bakery products, a fat replacer in dairy and meat products, hydrogels, adduct in packaging films and pharmaceutical products such as wound dressings (Courtin & Delcour, 2002). In addition, AX can help to maintain regularity of colonic functions and can possibly contribute to human health by reducing the risk of chronic diseases (Gibson, Beatty, Wang, & Cummings, 1995). Therefore, AX, as a kind of newly found bioactive constituent, has become a hot topic in the research field. Nevertheless, up to now, most AXs are obtained from cereals and limited studies have been carried out on AX from rhizomes.

As one of important rhizome plants, *Canna edulis* Ker, belonging to the genus *Canna* (Cannceae), is largely cultivated in South America, Vietnam, Thailand and China (Chansri, Puttanlek, Rungsardthong, & Uttapap, 2005). The dry rhizome of *C. edulis* contains 70–80% starches which are reported more digestible than other kinds of starches (Pérez, Lares, & González, 1997). In the process of starch extraction, abundant residues discarded as waste are potentially environmental problems because they are highly susceptible to putrefaction. In our preliminary experiment, we found some polysaccharides showed the characteristics of AX. Therefore, it is of great significance to study the AX from the by-product of C. *edulis* and explore their bioactivities in order to turn the by-product into value-added materials.

The study of the polysaccharides as food ingredient needs to explore their function on digestive enzymes. We chose the available and well-recognized in *vitro* models concerning digestion such as β-lactoglobulin (β-lg) digestion and tributyrin (TBG) hydrolysis as the first step for bioactivity assay. Several studies have demonstrated that the presence of soluble polysaccharides reduces protein digestibility in *vitro* and in *vivo* (Astwood & Morris, 1992; El Kossori et al., 2000; Eggum, 1995; Larsen, Wilson, & Moughan, 1994). The effects of several soluble fibers on the hydrolysis of tributyrin (TBG), under conditions that relate to physiological conditions, have been assessed in *vitro* study recently (O'Connor, Sun, Smith, & Melton, 2003). It has also been reported that different types of dietary fiber have the capacity to inhibit the activity of pancreatic lipase (Hendrick, Tadokoro, Emenhiser, Nienaber, & Fennema, 1992). So AX may exert influence on the digestion of β-lg and the hydrolysis of TBG.

Therefore, the objective of this work was to recycle and utilize the by-product of *C. edulis* after the extraction of starch. AXs were extracted and isolated from the by-product, and their structures

* Corresponding author. Tel.: +86 021 66137037; fax: +86 021 66137037.
E-mail addresses: JuanZhang@shu.edu.cn (J. Zhang), zhengwuwang@sjtu.edu.cn (Z.-W. Wang).

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were characterized by GC, GC–MS and NMR. In order to develop the function of AXs, their influences on pepsin and trypsin enzymatic digestibility of β-lg and TBG hydrolysis by lipase were further determined.

2. Materials and methods

2.1. Samples and reagents

Fresh rhizomes of *C. edulis* were obtained from Guizhou Ziyun Jiahe Chemical Co. Ltd. in Guizhou Province of China. *C. edulis* by-product was obtained from residues of rhizomes after the extraction of starch. The by-product was washed with water several times and dried at ambient temperature for 24 h. The products were ground with a mortar and pestle to a fine powder passing through a 60 mesh sieve. The powder was transferred to airtight plastic bags and stored in a desiccator at room temperature for further analysis. Cellulase "Onozuka" R-10 was purchased from Yakult. Co. Ltd. (Japan). The other enzymes were from Sigma–Aldrich Chemical Co. Ltd. (USA). Deuterated water (D_2O), deuterated sodium borohydride ($NaBD_4$) and methyl iodide (CH_3I) were obtained from Sigma–Aldrich Chemical Co. Ltd. (USA). N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC) was obtained from Fluka & RDH Co. Ltd. (Switzerland). All other reagents were of chromatographic or analytical grade quality.

2.2. Extraction and purification

AX was extracted according to the method of Knutsen and Holtekjoen (2007) with slight modification. The powder of *C. edulis* by-product was subjected to alkali extraction with 1 M NaOH with 1% $NaBH_4$ (w/v) at room temperature for 30 min. After centrifugation, the base-soluble polysaccharides were precipitated by adding equal volume of isopropanol, washed twice with 60% isopropanol and once with pure isopropanol, then resuspended in water, dialyzed and freeze-dried, giving crude AX.

In order to purify crude AX, the coextracted starch was first degraded by α-amylase (A3176, Type VI-B, ≥10 units/mg solid, from porcine pancreas, Sigma) at 37 °C in 0.2 mM phosphate buffer at pH 6.9 and then pancreatin (A0585, from porcine pancreas, Sigma) (200 mg)/amyloglucosidase (100 μL) (A3306, from *Aspergillus niger*, Sigma) enzyme mixture at pH 5.2 in a shaking water bath at 37 °C. Then the mixture was treated with cellulase ("Onozuka" R-10, Yakult. Co. Ltd., Japan) at 40 °C to remove the coextracted glucan. The degradated products were removed by precipitation of the remaining polysaccharides with two volumes of isopropanol and subsequent washing with 60% isopropanol and dialysis. The purified AX was obtained after freeze-drying.

Furthermore, AX (50 mg) extracted above were dissolved into 0.4 M NaOH aqueous solution. The sample solution was separated repeatedly by gel column chromatography on Sepharose CL-4B and ion-exchange column chromatography on DEAE-Sephadex A-50, to obtain AXI and AXII (Fig. 1).

2.3. Structural characterization

2.3.1. Single sugar composition

The samples were hydrolyzed, and the released sugars were transformed into alditol acetates with acetic anhydride in the presence of 1-methylimidazol according to the method of Blakeney, Harris, and Stone (1983). Quantification was performed in a Shimadzu GC-2010 gas chromatograph (Shimadzu Co., Kyoto, Japan) equipped with a hydrogen flame ionization detector. The column used was Rtx-5 (30 m × 0.25 mm × 0.25 μm) and nitrogen served as carrier gas. Temperatures of injector and detector were 290 °C and 280 °C, respectively. An oven temperature program of initial temperature 100 °C with a hold of 5 min, followed by a temperature rise of 20 °C/min to 190 °C, 3 °C/min to 260 °C, and 10 °C/min to 280 °C with a final hold of 5.17 min was conducted. Data were collected and processed with an Agilent Chem Station software system (Agilent Technologies, Waldrom, Germany) and β-D-allose was used as an internal standard.

2.3.2. Methylation

Reduction of uronic acids was carried out according to method of Taylor and Conrad (1972) with modification. In brief, 5 mg of samples were dissolved in 5 mL of D_2O and 15 mg of EDC was added into the solution. The reaction was allowed to proceed for at least 2 h. Then 15 mg of $NaBD_4$ was added slowly to the reaction mixture at room temperature. After 4 h, the reacted solution was dialyzed against distilled water and then lyophilized.

The methylation of the reduced sample was carried out according to the method of Ciucanu and Kerek (1984). The sample (4–5 mg) was dissolved into DMSO (0.3–0.5 mL). Then the solution was added finely powdered NaOH (20 mg) and CH_3I (0.1 mL). The mixture was stirred (1000 × *g*) for 6 min in a closed vial at 25 °C. Then H_2O (1 mL) and $CHCl_3$ (1 mL) were added into the reaction solution, and the $CHCl_3$ layer was washed with water (3 × 10 mL) and dried.

2.3.3. Preparation of partially O-methylated alditol acetates and GC–MS examination

The reaction product was hydrolyzed with 2 M H_2SO_4 for 1 h at 100 °C. The released partially O-methylated aldoses were transformed into alditol acetates with acetic anhydride in the presence of 1-methylimidazol according to the method of Blakeney et al. (1983). Quantification was performed in an AutoSystem XL GC/TurboMass MS Gas Chromatography/Mass Spectrometry (Perkin Elmer Co., USA). The column used was Rtx-5 (30 m × 0.25 mm × 0.25 μm) and nitrogen served as carrier gas. Temperatures of injector and detector were 290 °C and 280 °C, respectively. An oven temperature program of initial temperature 100 °C with a hold of 5 min, followed by a temperature rise of 20 °C/min to 190 °C and 3 °C/min to 260 °C, and 10 °C/min to 280 °C with a final hold of 5.17 min was conducted.

2.4. Nuclear magnetic resonance spectroscopy

The ^1H NMR and ^{13}C NMR data were measured on an Avance III 400 spectrometer (Bruker Co., Switzerland) at 400 MHz for ^1H and 100 MHz for ^{13}C. The chemical shifts (δ) are reported in ppm downfield from tetramethylsilane (TMS) using TMS as standard. The fraction of arabinose substitution patterns of xylose, including $F_{mono\ 3\text{-}O}$, $F_{mono\ 2\text{-}O}$, $F_{mono\ 2,3\text{-}di\text{-}O}$ and $F_{unsubstituted}$, were calculated according the method of Knutsen and Holtekjoen (2007).

2.5. Influence on β-lactoglobulin digestion in vitro

2.5.1. Preparation of β-lactoglobulin powder/sample mixtures

Mixtures of β-lg powder and samples were prepared according to previous report with modification (Nacer, Sanchez, Villaume, Mejean, & Mouecoucou, 2004). The mixtures of β-lg powder and samples were prepared to obtain 0 (β-lg without sample), 1, 10, 20, 30, and 50% of relative sample concentration. Benzoic acid (0.25%) was added to all of the solutions to prevent bacterial contamination.

2.5.2. Peptic digestion

One milliliter of pepsin (800–2500 U/mg protein, 1:10,000) in 0.02 M HCl (6 mg/mL) was added to 15 mL of mixtures. For simulating the in *vivo* gastric digestion, the pH of dispersions was progressively reduced from pH 7 to 2 within 2 h by adding 0.02 M

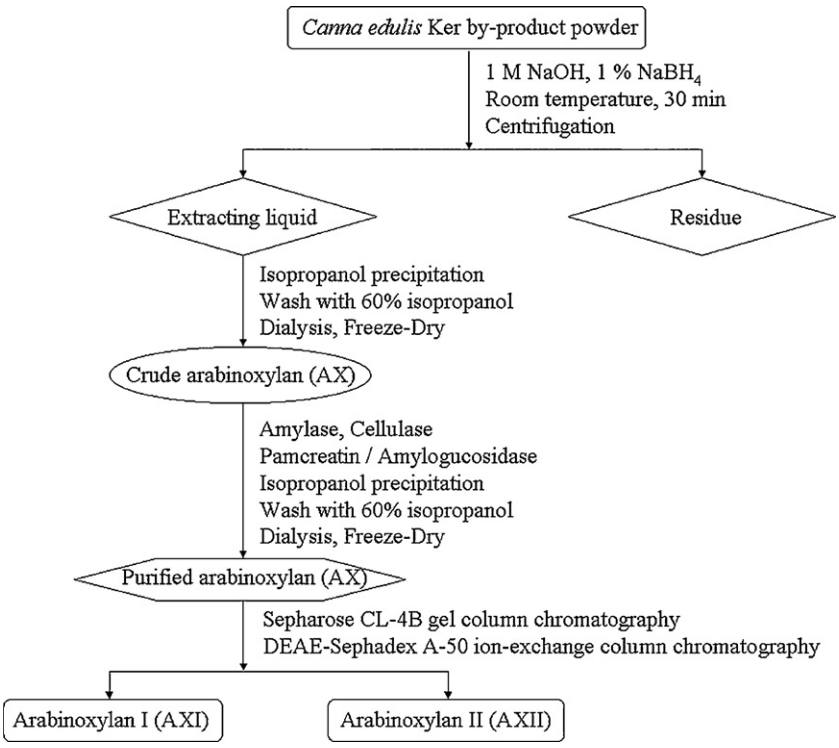

Fig. 1. The scheme of extraction and purification of *Canna edulis* Ker arabinoxylan I and II.

HCl at 37 °C. The digestion was stopped at pH 5, 4, 3 and 2 by adding 30% (v/v) trichloroacetic acid (TCA). Samples were centrifuged and 10 mL of supernatant were taken for soluble nitrogen analysis.

2.5.3. Digestion in dialysis bags

The in *vitro* total digestion of the mixtures was carried out at 37 °C in a dialysis cell according to method of Mouécoucou, Villaume, Sanchez, and Méjean (2004) with modification. Peptic digestion of the mixtures was made as described in the above section. Peptic digestion was stopped by raising the pH to 8 with 2 M NaOH. Then, the samples were transferred to dialysis bags with MWCO 8000 or 1000 Da which were placed in 100 mL sodium phosphate buffer (0.01 M, pH 8). Then, 1 mL of trypsin (>250.N.F.u/mg 1:250) at the concentration of 10 mg/mL in the same sodium phosphate buffer was added. Digestion products (1 mL) diffusing through the dialysis bag were collected every hour for 6 h and the fractions were taken for soluble nitrogen analysis.

2.5.4. Enzymatic activity

The influences of samples on pepsin and trypsin enzymatic activities were measured using bovine haemoglobin (Hb) and BAPNA as substrates according to the methods described by Ryle (1984) and Kakade, Rackis, McGhee, and Puski (1974), respectively.

2.6. Influence on tributyrin hydrolysis in vitro

Influence of samples on lipase hydrolysis of TBG was carried out according to the method of O'Connor et al. (2003). Sample was suspended in Bis–Tris buffer (50 mM, pH 6.5), sonicated for 1 min, and stored at 4 °C for 24 h before use. The standard emulsion was prepared by dispersing sodium caseinate (2.4 g) and L-α-lecithin (200 mg) in 100 mL Milli-Q water with a magnetic stirrer, before adjusting the volume to 200 mL by adding water. This preparation was stirred vigorously until all the solids were dissolved and the emulsion was used within 3 days. Enzymatic solution was prepared through solubilization of lipase [from porcine pancreas Type II, 100–400 units/mg protein (using olive oil), 30–90 units/mg protein (using triacetin)] in the Bis–Tris buffer. The total volume of the initial titration solution was 50 mL and was made up as follows: substrate emulsion, obtained by adding substrate TBG (100 mg) into the casein–lecithin emulsion (25 mL), various samples (2, 4, 6, 8, 10 g/L, 25 mL) or Bis–Tris buffer (25 mL, control sample), and the enzyme solution (100 μL, 5 mg/mL). During the titration, the temperature was maintained at 37 °C and the pH at 6.5. The pH of all samples was readjusted to 6.5 before lipase addition. Then, the enzyme solution was added, the titrator was activated, and the volume of NaOH required maintaining pH 6.5 was recorded automatically. Relative activity of lipase was calculated.

2.7. Statistical analysis

All determinations were triplicates, and mean values and standard deviations were calculated. Analysis of variance (ANOVA) was performed and the mean separation was done by LSD ($P \leq 0.05$) using SPSS 13.0 program for windows (SPSS Inc., IL, USA).

3. Results and discussions

3.1. Structural determination

After enzymatic treatment, the purified AX was separated repeatedly by column chromatography, leading to the isolation of two single components named AXI and AXII. As shown in Table 1, for two samples, arabinose (A) and xylose (X) are the major constituents, with a small quality of glucuronic acid (GlcA). Based on the arabinose/xylose ratio calculated from the GC analyses (Table 1), it is clear that the ratio is higher for AXI compared to that of AXII, which indicates a higher arabinose substitution of xylose backbone in the AXI.

AXI and AXII were per-O-methylated and the distribution of the corresponding methyl ethers is shown in Table 2. The trace of terminal 2,3,4-Me_3Xyl and high amount of 2,3-Me_2Xyl, 2-MeXyl

Table 1
Single sugar composition and the fraction of substitution patterns of xylose of arabinoxylan I and II (%).

Variety	AXI (%)	AXII (%)
Arabinose	33.80 ± 0.55 a	24.14 ± 0.72 b
Xylose	55.61 ± 0.33 a	71.13 ± 1.06 b
Glucuronic acid	11.22 ± 0.16 a	5.52 ± 0.14 b
Arabinose/xylose ratio	0.61 ± 0.01 a	0.34 ± 0.03 b
$F_{mono\ 3\text{-}O}$	0.61	0.56
$F_{mono\ 2\text{-}O}$	0.24	0.36
$F_{mono\ 2,3\text{-}di\text{-}O}$	–	–
$F_{unsubstituted}$	0.15	0.08

Values are means ± S.D. Values not sharing a common letter are significant different $P < 0.05$.

and 3-MeXyl suggest that 1,4-linked xylose units as backbone are branched in positions 2 and 3 in both AXI and AXII with varying ratios. For two samples, arabinosyl and glucuronosyl residues both locate at side chain, which could be deduced from minor amount of terminal 2,3,5-Me_3Ara and 2,3,4-Me_3GlcA, respectively. In addition, in each case of AXI and AXII, the amount of branched xylose accords well with the fractions of substitution patterns of xylose ($F_{mono\ 3\text{-}O}$, $F_{mono\ 2\text{-}O}$, $F_{mono\ 2,\ 3\text{-}di\text{-}O}$ and $F_{unsubstituted}$) by combining arabinose/xylose ratio obtained from GC and the anomeric signals of arabinose in the δ 5.2–5.4 ppm region in the ^{1}H NMR spectra.

In order to determine the occurrence position of glucuronic acid at branched xylose, partial acid hydrolysis was carried out. Both AXI and AXII were dispersed in 12 mol/L oxalic acid aqueous solution and hydrolyzed at 100 °C for 7 h, respectively. The hydrolyzed liquid was concentrated and then added sodium hydroxide solution to attain 0.8 mol/L of its concentration in 1 mL of final volume. The sample solutions were separated by gel column chromatography on Sepharose CL-4B to give two peaks, respectively, namely, AXI-1 and AXI-2 from AXI and AXII-1 and AXII-2 from AXII. AXI-2 and AXII-2, the second peaks of each sample, were mainly consisted of arabinose as determined by GC after their acetylation. For the first peaks AXI-1 and AXII-1, the existence of 2-MeXyl was detected by GC–MS after their methylation. These results suggest that glucuronic acid occurs at branched xylose at position 2. Moreover, it can well be deduced that arabinose occurs at branched xylose at position 3, in view of the equal amounts of arabinosyl terminal and 2-MeXyl residue.

The ^{13}C NMR spectrum of AXI shows 15 carbon signals which are assigned as follows: δ 108.6 (A, C1), 103.4 (GlcA, C1), 102.6 (X, C1), 85.3 (X, C3; A, C4), 83.2 (X, C2), 82.7 (A, C2), 79.4 (X, C4), 77.9 (A, C3), 74.8 (X, C3; GlcA, C5), 73.8 (X, C2; GlcA, C2), 72.2 (GlcA, C3), 70.4 (GlcA, C4), 67.0 (GlcA, C6), 66.1 (X, C5), 62.6 (A, C5). The linkage site among xyloses is β (1 → 4), deduced from carbon signals [δ 102.6 (X, C1); 79.4 (X, C4)] which shifted to downfield in comparison with those in the standard spectrum of β-D-xylose [δ 97.5 (X, C1); 70.2 (X, C4)] (Agrawal, 1992). Moreover, two sets of different chemical shifts [δ 83.2 (X, C2) and 73.8 (X, C2); 85.3 (X, C3) and 74.8 (X, C3)] imply the existence of 2 and 3 branched xylose. Compared with standard spectrum of α-L-Arabinose [δ 101.9 (A, C1)], the signal [δ 108.6 (A, C1)] shifted to downfield, which suggests that arabinose is branched at position 1 (Bock, Pederson, & Pederson, 1984). It can well be deduced that glucuronic acid is also branched at position 1, in light of the signal [δ 103.4 (GlcA, C1)] shifting to downfield than that (δ 96.8) in the standard spectrum of glucose (Agrawal, 1992). Additionally, the ratios among the OMe-Alditol acetates (Table 2) can well be calculated as follows: [Ara (1→]:[→4)Xyl (1→]:[→3,4) Xyl (1→]:[→2,4) Xyl (1→]:[GlcA (1→] = 32.86:8.03:33.21:13.42:13.21 ≈ 4:1:4:2:2. It suggests that among average seven xylosyl residues, four residues are substituted by mono arabinosyl moiety at position 3 and two by mono glucuronosyl moiety at position 2 as side chains, when xylan is used as chief chain.

Table 2
Partially O-methylated alditol acetates from arabinoxylan I and II.

OMe-Alditol acetates	Linkage site	ASPI (%)	ASPII (%)
2,3,5-Me_3Ara	Ara (1→	32.86	25.63
2,3,4-Me_3Xyl	Xyl (1→	+	+
2,3-Me_2Xyl	→4) Xyl (1→	8.03	38.85
2-MeXyl	→3,4) Xyl (1→	33.21	24.86
3-MeXyl	→2,4) Xyl (1→	13.42	5.98
2,3,4-Me_3GlcA	GlcA (1→	13.21	6.13

The ^{13}C NMR spectrum of AXII also shows 15 carbon signals which are assigned as follows: δ 108.8 (A, C1), 103.1 (GlcA, C1), 102.7 (X, C1), 85.2 (X, C3; A, C4), 83.3 (X, C2), 82.6 (A, C2), 79.2 (X, C4), 77.8 (A, C3), 74.6 (X, C3; GlcA, C5), 73.4 (X, C2; GlcA, C2), 72.3 (GlcA, C3), 70.6 (GlcA, C4), 67.3 (GlcA, C6), 65.8 (X, C5), 62.3 (A, C5). Similar to those of AXI, the linkage site [β (1 → 4)] among xylosyl residues is deduced from carbon signals [δ 102.7 (X, C1); 79.2 (X, C4)], and their branched sites at positions 2 and 3 are confirmed by two sets of different chemical shifts [δ 83.3 (X, C2) and 73.4 (X, C2); 85.2 (X, C3) and 74.6 (X, C3)]. Both arabinose and glucuronic acid are branched at position 1, in view of the peaks located at δ 101.9 (A, C1) and 103.4 (GlcA, C1), respectively. Moreover, the ratios among the OMe-Alditol acetates (Table 2) can well be calculated as follows: [Ara (1→]:[→4)Xyl (1→]:[→3,4) Xyl (1→]:[→2, 4) Xyl (1→]:[GlcA (1→] = 25.63:38.85:24.86:5.98:6.13 ≈ 4:6:4:1:1. It suggests that among average eleven xylosyl residues, four residues are substituted by mono arabinosyl moiety at position 3 and one by mono glucuronosyl moiety at position 2 as side chains, when xylan is used as chief chain.

3.2. Influence on β-lactoglobulin hydrolysis in vitro

3.2.1. Peptic digestion

The effect of AX on the peptic digestibility of β-lg is exhibited in Table 3. The digestibility of β-lg alone is very low at all pH values, with only 1–3% of N release values, which is in accordance with pervious report (Nacer et al., 2004). It can be explained by β-lg sequence, which owns 50 bondings of potential peptic cleavage sites, however, most of those are buried in the hydrophobic core of the molecular and are not accessible (Chobert, Briand, Grinberg, & Haertle, 1995). The low digestibility of β-lg can also be ascribed to the aggregate formation of products of β-lg peptic hydrolysis that could render the access of pepsin to its cleavage bonds difficult. It was found that β-lg was intact after its passage through the stomach in *vivo* (Miranda & Pelissier, 1983). So the resistance of β-lg to peptic digestibility could also be attributed to its stable conformation at pH 2, resulting from increased internal hydrogen bonding that arises between either two titrated carboxyl groups or one amide and one carboxyl group (Kella & Kinsella, 1988).

With the increase of AX levels, N release gradually increased at all pH values ($P < 0.05$), in disagreement with those of low-methylated pectin, high-methylated pectin and amidated pectin (Nacer et al., 2004). N release maximized when AX's concentration reached to 50%, despite the reduction of pepsin enzymatic activity measured in the assay of enzymatic activity. It suggests that the increase of N release is not due to an increased ability of pepsin to hydrolyze β-lg but is due to an incomplete TCA precipitation of protein, as a result of interaction between AX and protein or the products of β-lg peptic hydrolysis. It appeared that the concentration increase of AX not only enhanced the repulsion of β-lg molecules, which promoted the protein solubility, but also favored the interactions, resulting in the unexpected increase of N release. What is more, an increase of N release related to the pH decrease is observed with different AX concentrations. These results agree with those reported for xylan (Mouécoucou et al., 2004). It indicates that β-lg can carry more positive charges at lower pH, and then the interaction between AX and β-lg strengthens.

Table 3
Influence of arabinoxylan on the peptic digestibility of β-lactoglobulin (%).

pH	Levels of soluble dietary fiber (%)					
	0	1	10	20	30	50
5	0.95 ± 0.05 a, A	0.99 ± 0.01 a, A	1.13 ± 0.03 b, A	1.32 ± 0.06 bc, A	1.44 ± 0.21 c, A	2.11 ± 0.18 d, A
4	0.95 ± 0.03 a, A	0.97 ± 0.03 a, A	1.09 ± 0.04 b, A	1.30 ± 0.05 c, A	1.45 ± 0.07 d, A	2.09 ± 0.09 e, A
3	1.15 ± 0.05 a, B	1.18 ± 0.04 a, A	1.34 ± 0.11 b, B	1.52 ± 0.07 c, B	1.84 ± 0.04 d, B	2.26 ± 0.11 e, A
2	1.49 ± 0.07 a, C	2.32 ± 0.08 bc, B	2.23 ± 0.07 b, C	2.46 ± 0.05 c, C	3.07 ± 0.06 d, C	3.39 ± 0.14 e, B

Values are means ± S.D. Values not sharing a common letter are significant different $P < 0.05$; first letter is related to row, the second to columns.

3.2.2. Tryptic digestion

Influence of AX on the tryptic digestibility of β-lg is exhibited in Fig. 2. It was found that tryptic digestibility of β-lg was higher in dialysis bag with MWCO 8000 Da than that with MWCO 1000 Da. The result suggests that the complex formed after β-lg digestion may be consisted of some peptide chains between 8000 and 1000 Da and small peptides of less than 1000 Da.

Compared to those of β-lg alone, N release contents from AX/β-lg mixtures are lower, in accordance with the results in the previous reports (Astwood & Morris, 1992; Eggum, 1995; Lamghari et al., 2000; Larsen et al., 1994). The reduction of N release by AX after 6 h of tryptic digestion was 8.91% and 10.48% through the dialysis bags with MWCO 1000 and 8000 Da, respectively. In light of no influence of AX on trypsin activity, which was confirmed in the measurement of enzymatic activity, the reduction of N release cannot be attributed to the reduction of enzymatic activity. As AX is a neutral oligosaccharide, the reduction of tryptic digestibility of β-lg could possibly be explained by the non-ionic interaction effect between AX and protein. Moreover, AX and β-lg are similarly charged and electrostatic interactions between these biopolymers do not occur at pH 8. Another possible reason is that electrostatic interactions occur between AX and positively charged peptides, as arginine and lysine, released by trypsin hydrolysis.

As observed in Fig. 2, with the increase of AX ratios, the contents of N release gradually decreased, which can be ascribed to increased non-ionic interaction effect between AX and β-lg or electrostatic interaction between AX and positively charged peptides in the hydrolyzed solution. Moreover, with the prolonged time, N release contents increased, indicating that β-lg was gradually hydrolyzed by trypsin. Additionally, the rate of N release gradually reduced with the extension of hydrolysis time.

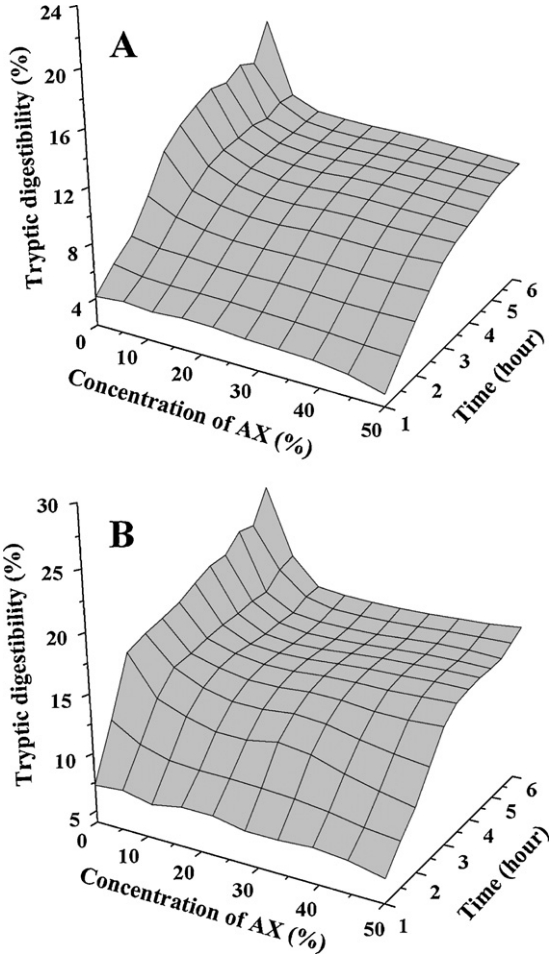

Fig. 2. Influence of arabinoxylan on the tryptic digestibility of β-lactoglobulin (A: MWCO 1000 Da; B: MWCO 8000 Da).

3.2.3. Enzymatic activity

3.2.3.1. Gastric pepsin activity. When the concentration of AX was 0, 1 and 50%, the corresponding enzymatic activity was 1.27, 1.12 and 0.48 μmol/min, respectively. It suggests that the addition of AX can inhibit pepsin enzymatic activity to some extent, similar to those of viscous polysaccharides (Larsen et al., 1994; Shah, Atallah, Mahoney, & Pellet, 1982). Reduction of enzymatic activity can be attributed to direct interaction between AX and enzyme, or inhibition of AX on binding of enzyme and substrate. Therefore, the increase of N release with the addition of AX seems to be due to insoluble β-lg-AX aggregates and not an increase of β-lg pepsin hydrolysis.

3.2.3.2. Trypsin activity. It was found that AX had no obvious effect on trypsin activity, which is in accordance with the results reported for gum arabic and xylan (Mouécoucou et al., 2004).

3.3. Influence on tributyrin hydrolysis in vitro

Fig. 3 shows the inhibitory effects of AX with five concentrations on the activity of lipase against TBG. The initial rates of TBG hydrol-

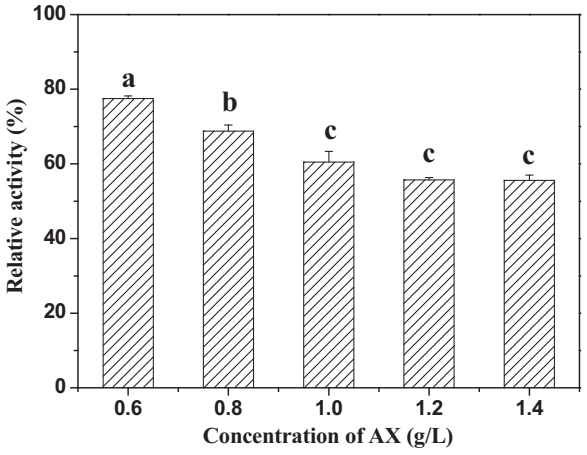

Fig. 3. Inhibitory effect of arabinoxylan on the activity of lipase. Values not sharing a common letter are significant different $P < 0.05$.

ysis in all reactions were determined within the initial linear region of the titration curves. In agreement with those reported for CMC, pectin, carrageenan and gum Arabic (O'Connor et al., 2003), AX had an obvious inhibition effect on lipase activity. It can be inferred that AX increases the droplet size of the emulsion and decreases the interfacial area of the generated emulsion, resulting in decreased contact between the enzyme and the substrate and the reduction of TBG hydrolysis (Lairon, 1997; Pasquier et al., 1996). In addition, the inhibition effect increased with the increase of AX content.

With the increase of AX concentration, enzymatic activity gradually decreased. It has been reported that the changes in viscosity modulated the effects of dietary fiber, including enzyme activity (Dukehart, Dutta, & Vaeth, 1989). So we could infer that viscosity is a major contribution to the reduction in the rate of lipolysis and it may affect the distribution of the hydrophobic substrate and TBG. Moreover, the increase of viscosity may reduce contact between the enzyme and the substrate, resulting in the decrease of enzymatic activity.

4. Conclusions

AXs were separated and purified from *C. edulis* by-product and their structures were characterized by GC, GC–MS and NMR. Moreover, the influence of AX on pepsin and trypsin digestibility of β-lg was studied at different AX levels. Furthermore, the effect of AX on TBG hydrolysis by lipase was discussed. The results showed that AX could inhibit enzymatic digestibility of β-lg and TBG hydrolysis by lipase to some extent. It is beneficial for the development of *C. edulis* AX as a functional food ingredient.

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