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Thermal analysis and physiological behavior of cellulose/pectin complex from *Canna edulis* Ker by-product

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ABSTRACT

Water-soluble extract (WSE), chelated-soluble extract (CSE) and acid-soluble extract (ASE) were obtained from *Canna edulis* Ker by-product, and their thermal characteristics and physiological behavior were studied. Thermal properties of WSE and ASE demonstrated the exiting of cellulose, as main constituents in the extracts. Different from those of WSE and ASE, the thermal characteristics of CSE demonstrated that it was chiefly composed of cellulose and pectin. The three extracts inhibited both gastric pepsin and lipase enzymatic activities to some extent, and tryptic digestion of β-lactoglobulin as well as lipase hydrolysis of tributyrin in vitro. Therefore, the three extracts could be used as additives in the food industry, in view of safety of *C. edulis* by-product confirmed by animal experiment.

1. Introduction

Canna edulis Ker belonging to the genus *Canna* (Cannceae) is largely cultivated in South America, Vietnam, Thailand and China. The dry rhizome of *C. edulis* contains 70–80% starches which are reported more digestible than other kinds of starches (Chuenkamol, Puttanlek, Rungsardthong, & Uttapap, 2007). *C. edulis* residue, as waste discarded after starch extraction, is a potentially environmental problem because that it is highly susceptible to putrefaction as a result of high moisture content (80%). To the best of our knowledge, this by-product might contain plenty cellulosic and pectic polysaccharides which can be used in food industry in view of safety confirmed by animal experiment (Jun, Jo, Hwangbo, Lee, & Imai, 2006). Consequently, it is of significance to exploit this by-product, considering that the utilization could not only eliminate a possible source of pollution but also increase economic value.

Cellulosic polysaccharides are composed of various organic materials (primarily cellulose, hemicellulose and lignin) and their thermal treatment leads to a variety of physical and chemical changes. Pectic polysaccharides include the galacturonans (homogalacturonan, substituted galacturonans, and RG-II) and rhamnogalacturonan-I (Caffall & Mohnen, 2009). It has been proved that the increased intake of cellulosic and pectic polysaccharides benefits a number of gastrointestinal disorders, such as gastroesophageal reflux disease, duodenal ulcer, diverticulitis, constipation and hemorrhoids (Anderson et al., 2009). On the basis of these, natural extracts including cellulosic and pectic polysaccharides have been extensively used as food additive in the production of biscuit, bread and cracknel so on.

It is well-known that most foods are subjected to variations in their temperature during production, transport, storage, preparation and consumption. Temperature changes cause alterations in the physical and chemical properties of food components which influence the overall properties of the final product, e.g., taste, appearance, texture and stability. Therefore, it is important to determine thermal characteristics of polysaccharides as food additives.

It has been demonstrated that the presence of polysaccharides reduces protein digestibility in vitro and in vivo (Astwood & Morris, 1992; Eggum, 1995; El Kossori et al., 2000; 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 (O'Connor, Sun, Smith, & Melton, 2003). It has also been reported that dietary fiber can inhibit the activity of pancreatic lipase (Hendrick, Tadokoro, Emenhiser, Nienaber, & Fennema, 1992). So natural extracts of *C. edulis* may have an impact on the digestion of β-lactoglobulin (β-lg) and the hydrolysis of TBG, which were generally used to evaluate physiological behaviors of natural extracts.

In our study, thermal characteristics of three different kinds of natural cellulosic/pectic polysaccharides from *C. edulis* were determined by STA 409 C Thermal Analysis. Moreover, physiological behaviors of the natural extracts were evaluated using the models of the digestion of β-lg and the hydrolysis of TBG. Additionally,

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their influences on enzymatic activities, including pepsin, trypsin and lipase, were studied.

2. Materials and methods

2.1. Samples and reagents

Fresh rhizomes of *C. edulis* were obtained from Guizhou Ziyun Jiahe Chemical Co. Ltd in the 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. All other reagents were of chromatographic or analytical quality.

2.2. Extraction and purification

C. edulis by-product (10 g) was added into distilled water (300 mL) adjusted pH to 5.0 with HCl and NaOH solution. The mixture was extracted through refluxing at 80 °C for two times and the filtrated liquids was concentrated at 50°C under reduced pressure. The concentrate obtained was purified through further dialysis against distilled water with four changes for 24 h and the dialysate was freeze-dried to give water-soluble extract (WSE). Similar to WSE, chelated-soluble extract (CSE) and acid-soluble extract (ASE) were extracted and purified using 0.5% oxalate ammonium and dilute HCl solution, respectively.

2.3. Thermal analysis

The simultaneous thermal analysis (differential scanning calorimetry DSC and thermogravimetry TG) was carried out using a STA 409 C-device (Netzsch, Selb, Germany) according to Gloyna and Kunzek (1998), using the following conditions: linear heating rate 10 K/min from 20 to 550 °C, dynamic inert nitrogen atmosphere (75 mL/min), empty crucible as reference, sample weight approximately 10–20 mg. All runs were performed at least in duplicate. The extrapolated onset, peak and offset temperatures as well as the maximum heat flow E_{max} and the maximum degradation velocity v_{max} were calculated with the Netzsch software as shown in Einhorn-Stoll, Kunzek, and Dongowski (2007).

2.4. Influence on the digestion of β-lactoglobulin (β-lg) in vitro

2.4.1. Preparation of β-lactoglobulin (β-lg) 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.

2.4.2. 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 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 supernatants were taken for soluble nitrogen analysis.

2.4.3. 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 3000 or 14,000 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.

2.4.4. Enzymatic activity

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

2.5. Influence on lipase hydrolysis of tributyrin (TBG) 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 min incubation), 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 fiber-containing 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.

2.6. 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. Thermal analysis

WSE, CSE and ASE were obtained through extraction using water, oxalate ammonium and dilute HCl solution, respectively. In the TG spectrum (Fig. 1), the first degradation peak between 70 °C and 150 °C with a maximum at about 100 °C results from water release. It allows a determination of water content in the samples by a calculation of the weight loss in the TG signal. It was found that water content was between 7 and 8% in the three samples.

For WSE and ASE, in the exception of the degradation peak located at near 100 °C, the TG curves only showed one degradation

Table 1
Thermal analysis of extract from *Canna edulis* Ker by-products.

Sample	Release of humidity (DSC)					Pectin degradation			Cellulose degradation			Extract
	T_{on} (°C)	T_p (°C)	ΔT (°C)	E_{max} (mW/mg)	T_{on} (°C)	ΔT (°C)	Changed masses (%)	v_{max} (%/min)	T_{on} (°C)	ΔT (°C)	Changed masses (%)	v_{max} (%/min)	Changed masses (%)
WSE	58.3	100.5	48.4	0.73	–	–	–	–	290.8	39.4	−67.38	−15.13	−75.72
CSE	67.1	113.3	40.0	0.88	220.1	33.5	−24.37	−5.28	294.5	42.1	−34.09	−5.14	−68.17
ASE	63.3	100.6	57.6	0.81	–	–	–	–	294.7	39.3	−68.32	−17.93	78.22

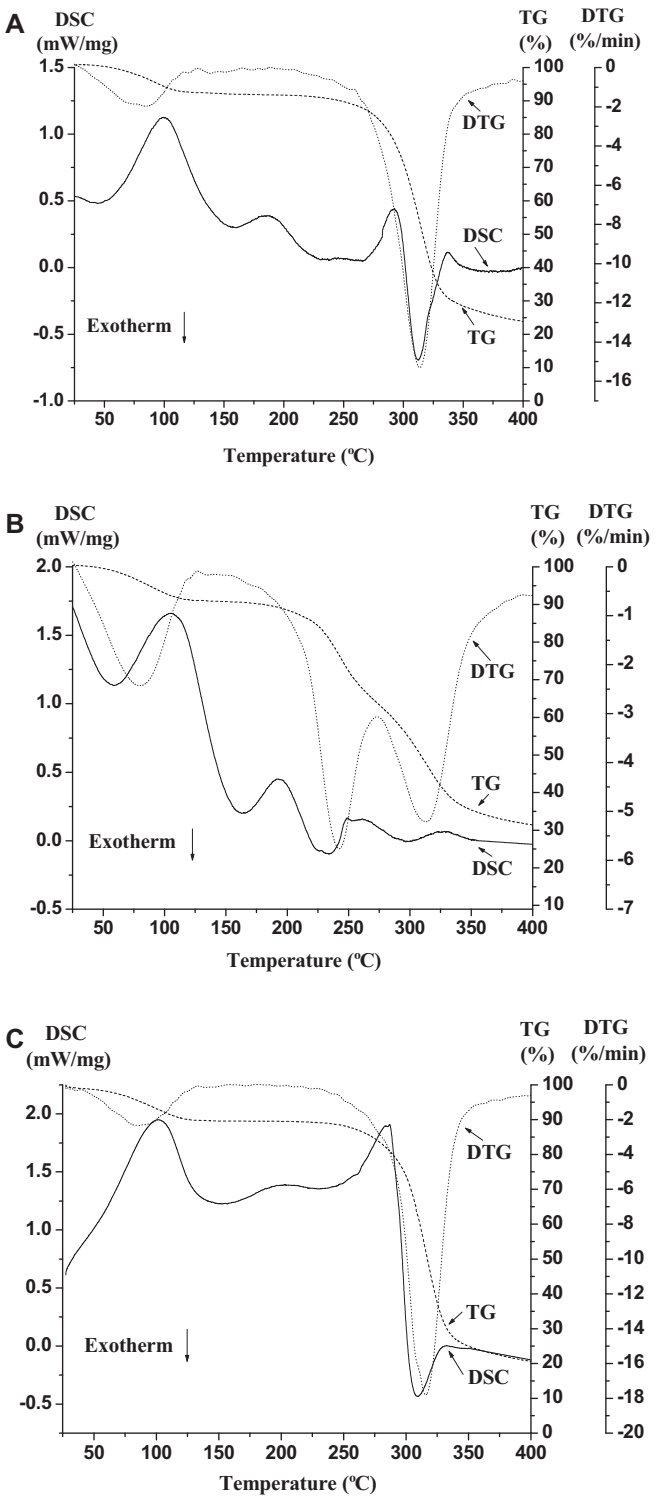

Fig. 1. Thermograms of water-soluble extract (A), chelating-soluble extract (B) and acid-soluble extract (C).

peak at about 310 °C, which can be attributed to the degradation of cellulose network. It has been confirmed that the degradation of cellulose is composed of a set of concurrent and consecutive reactions. As an important intermediate, levoglucosan goes undetected. The final breakdown products including C, CO, CO_2, H_2O, and combustible volatiles can be detected. These combustible volatiles and the carbon are oxidized in air in two stages represented as flaming combustion and glowing combustion, respectively. The process of

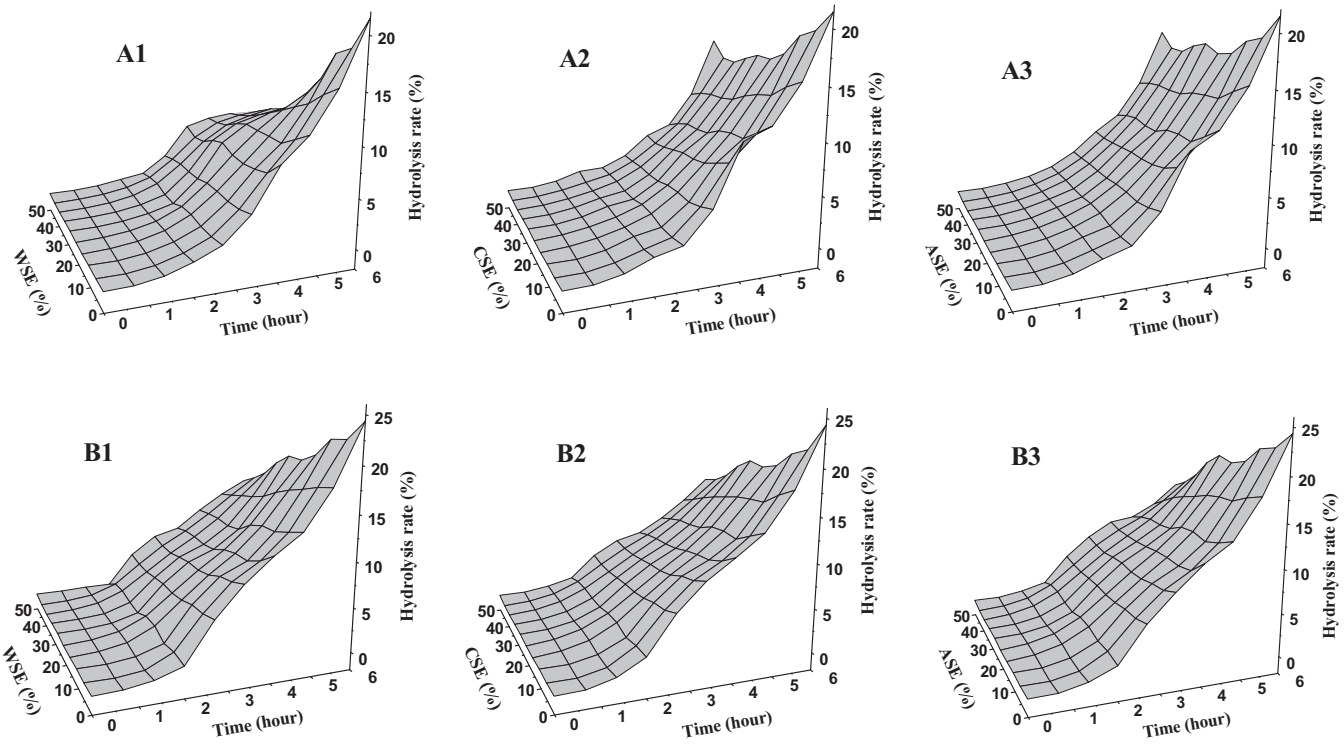

Fig. 2. Influence of water-soluble extract (1), chelating-soluble extract (2) and acid-soluble extract (3) on the tryptic digestibility of β-lactoglobulin with different MWCO 1000 Da (A) and 8000 Da (B).

combustion converts an endothermic degradation into an overall exothermic process (Lerdkanchanaporn, Dollimore, & Alexander, 1998).

Different those from WSE and ASE, another degradation peak with peak temperature of 242 °C exhibited pectic characteristics in the DTG curve of CSE (Zaleskaa, Ringb, & Tomasika, 2000). It has been reported that the pectin degradation is an exothermic reaction and it starts at temperatures of about 200 °C and ends at about 240–280 °C, depending on the molecular parameters, degree of modification and physical state (Einhorn-Stoll et al., 2007).

Three kinds of samples showed intricate thermal characteristics. In the DSC spectrum, except for the endothermic peak at about 100 °C resulting from water release, WSE and ASE exhibited two endothermic peaks and one exothermic peak. Among of them, the endothermic peak located at near 290 °C and the exothermic peak with the peak temperature of about 310 °C could result from combustion of final breakdown products of the cellulose including C, CO, CO_2, H_2O, and combustible volatiles (Lerdkanchanaporn et al., 1998). However, CSE showed three endothermic peaks as a result of combined action of cellulose and pectin. It indicates the complexity of constituents in the three samples.

Compared to those of WSE and CSE, ASE presented the maximum changed mass and degradation velocity (v_{max}) as well as smallest DTG peak width (ΔT) in the process of cellulose degradation, indicating that the degradation reaction of ASE is rapid and drastic (Table 1). It suggests that ASE is more homogenous according to Einhorn-Stoll et al. (2007) and Einhorn-Stoll and Kunzek (2009), which can be explained by some of the neutral sugar side chains cleaved in the process of dilute acid extraction (Pilnik & Voragen, 1992). Furthermore, the cellulose degradation of ASE started later in comparison with those of WSE and CSE, representing the highest thermal stability for ASE, in view of the extrapolate onset temperature (T_{on}) which is an indicator of the thermal stability of the extract.

As shown in Fig. 1, for WSE and ASE, the DSC signal at first endothermic peak followed by typical exothermal degradation peak in the course of cellulose degradation, similar to HMP-W reported by Einhorn-Stoll and Kunzek (2009). Moreover, the endothermic DSC-peak started earlier than the exothermal DTG-peak, especially for ASE (earlier about 27 °C), that is, energetic effects were tested earlier than the weight loss. It can be attributed to conformation transformation before the degradation weight loss, which requires more energy than that released by the beginning thermal degradation. The conformation change might be the transformation from the stable $^4C_1$ chair conformation of the glucose ring via a $^{1,4}B$ conformation to the inverse $^1C_4$ chair conformation that has higher free energy *G*.

3.2. Influence on hydrolysis of β-lactoglobulin in vitro

3.2.1. Peptic digestion

The effects of WSE, CSE and ASE on the peptic digestibility of β-lg are exhibited in Table 2. The digestibility of β-lg alone was very low at all pH values, with only 1.0–2.5% of N release values 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).

For WSE and ASE, with the increase of their levels, N release gradually increased at all pH values, and the maximum values were obtained when the extract contents attained to 50%. In view of the reduction of pepsin enzymatic activity obtained in the measurement of enzymatic activity, it can be well deduced that the incomplete TCA precipitation of protein led to the increase of N release, as a result of interaction between cellulose and protein. Moreover, the interaction gradually strengthened as the concentration of the extract increased, resulting in the rising N release.

Table 2
Influence of water-soluble extract, chelating-soluble extract and acid-soluble extract on the peptic digestibility of β-lactoglobulin (%).

pH	Concentration of the extract (%)					
	0	1	10	20	30	50
WSP						
5	1.00 ± 0.01 a, A	1.02 ± 0.01 a, A	1.14 ± 0.04 b, A	1.30 ± 0.01 c, A	1.55 ± 0.03 d, A	2.15 ± 0.04 e, A
4	1.29 ± 0.01 a, B	1.32 ± 0.01 b, B	1.47 ± 0.02 c, B	1.62 ± 0.01 d, B	1.91 ± 0.01 e, B	2.60 ± 0.01 f, B
3	2.03 ± 0.01 a, C	2.06 ± 0.01 a, C	2.46 ± 0.04 b, C	2.49 ± 0.03 b, C	2.86 ± 0.03 c, C	3.46 ± 0.02 d, C
2	2.26 ± 0.03 a, D	2.33 ± 0.02 b, D	2.50 ± 0.04 c, C	2.71 ± 0.03 d, D	2.83 ± 0.02 e, C	3.83 ± 0.02 f, D
CSP						
5	1.00 ± 0.01 a, A	1.00 ± 0.01 a, AE	1.11 ± 0.02 b, A	1.17 ± 0.04 c, AG	1.47 ± 0.01 d, D	2.16 ± 0.06 e, A
4	1.35 ± 0.04 a, E	1.06 ± 0.01 b, BF	1.14 ± 0.06 c, A	1.14 ± 0.02 c, E	1.24 ± 0.02 d, E	2.36 ± 0.01 e, E
3	2.03 ± 0.02 a, C	2.42 ± 0.04 b, G	2.50 ± 0.02c, C	2.40 ± 0.03 b, F	2.35 ± 0.05 b, F	2.14 ± 0.07 d, A
2	2.25 ± 0.05 a, D	2.52 ± 0.02 b, H	2.11 ± 0.02 c, D	2.51 ± 0.01 b, C	2.54 ± 0.04 b, G	3.20 ± 0.05 d, F
ASP						
5	1.00 ± 0.01 a, A	1.04 ± 0.02 a, A	1.15 ± 0.06 b, A	1.24 ± 0.05 c, G	1.43 ± 0.02 d, D	1.97 ± 0.01 e, G
4	1.29 ± 0.02 a, B	1.18 ± 0.02 b, I	2.24 ± 0.04 c, E	2.43 ± 0.05 d, B	2.56 ± 0.04 e, G	3.12 ± 0.04 f, F
3	2.01 ± 0.02 a, C	2.19 ± 0.09 b, J	2.71 ± 0.02 c, F	2.81 ± 0.01 d, H	3.30 ± 0.02 e, H	4.69 ± 0.03 f, H
2	2.31 ± 0.01 a, F	2.61 ± 0.02 b, K	2.89 ± 0.07 c, G	3.02 ± 0.02 d, I	3.51 ± 0.02 e, I	5.07 ± 0.12 f, I

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

Different from those of WSE and ASE, N release in the CSE varied complicatedly along with the increase of the concentration. It could be attributed that the interactions between protein and cellulose or/and pectin, main constituents in the CSE demonstrated in the measurement of thermal analysis. Furthermore, according to the rate of digestibility, three extract for investigation were placed in the relative order of influence: CSE > WSE > ASE.

It is observed that the increase of N release was related to the pH decrease for the three extract with different concentrations. Additionally, the rate of N release was lower in the period with low pH values varying from 2 to 3, compared to that with high ones changing from 3.5 to 5. It indicates that decreasing pH value strengthened the interaction between extract and β-lg. Moreover, the resistance of β-lg to peptic digestibility can be also 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).

3.2.2. Tryptic digestion

The hydrolysis of β-lg was performed in dialysis bag with MWCO 1000 and 8000 Da under the condition of existence of WSE, CSE and ASE at different concentration. Fig. 2 exhibits the effect of three extract on the tryptic digestibility of β-lg. For the three extracts, N release was not detected within 1 h, attributing to low release content. In the comparison with those of β-lg alone, N release content from the mixture of the extract and β-lg was lower, suggesting that the addition of WSE, CSE and ASE inhibited β-lg hydrolysis. This result corresponded well with previous reports related to other proteins in vivo and in vitro (Astwood and Morris, 1992; Eggum, 1995; Lamghari et al., 2000; Larsen et al., 1994). In the sight of no influence of the three extract on trypsin activity measured below, the reduction of N release could be attributed to non-specific interactions between molecular species in the mixture at the low concentration, or interaction between the extract and protein induced by the viscosity at the high concentration.

With the increase of the extract content, N release content gradually decreased, which can be ascribed to strengthened interaction between the extract and protein arising from increased viscosity, resulting in the inhibition of β-lg hydrolysis. Moreover, N release content increased with the delay of hydrolyzed time, suggesting that β-lg was gradually hydrolyzed by trypsin. In addition, the three extract had a similar effect on β-lg hydrolysis by trysin.

For all the samples, N release content was different to some extent through the dialysis bag with MWCO 1000 and 8000 Da, and the content through the latter was higher than that through the former. This result indicates that the complex originated from β-lg digestion could be composed of some peptide chains between 1000 and 8000 Da and small peptides of less than 1000 Da.

3.2.3. Enzymatic activity

3.2.3.1. Gastric pepsin activity. As shown in Table 3, with the increasing concentration of WSE, CSE and ASE, gastric pepsin activity decreased, revealing that the addition of the extract had a negative effect on pepsin activity. CSE exhibited the strongest inhibition effect in comparison with those of WSE and ASE. The previous studies have also reported that viscous polysaccharides inhibited the digestive enzyme activity (Larsen et al., 1994; Shah, Atallah,

Table 3
Influence of water-soluble pectin, chelating-soluble pectin and acid-soluble pectin on pepsin enzymatic activity (μmol/min).

	Concentration (%)		
	0	10	50
WSE	1.27 ± 0.32 a, A	1.16 ± 0.05 b, A	0.61 ± 0.02 c, A
CSE	1.27 ± 0.17 a, A	1.14 ± 0.05 b, A	0.28 ± 0.09 c, B
ASE	1.26 ± 0.57 a, A	0.75 ± 0.12 b, B	0.42 ± 0.03 c, C

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

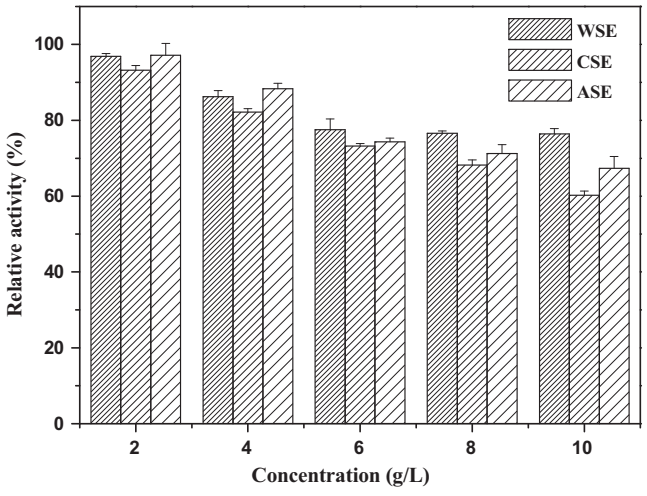

Fig. 3. Inhibitory effects of water-soluble extract, chelating-soluble extract and acid-soluble extract on the activity of lipase (%).

Mahoney, & Pellet, 1982). The reduction of enzymatic activity could be explained by direct interactions between extract and enzymes, or inhibition of extract on binding of enzyme and substrate.

3.2.3.2. Trypsin activity. It was found that trypsin enzymatic activities were not influenced by the addition of WSE, CSE and ASE, in accordance with those of gum arabic and xylan (Mouécoucou et al., 2004).

3.3. Influence on lipase hydrolysis of tributyrin in vitro

As exhibited in Fig. 3, WSE, CSE and ASE inhibited lipase activity against TBG to one degree or another, in agreement with the reports for other fibers including CMC, pectin, carrageenan and gum Arabic (O'Connor et al., 2003). According to the relative activity of lipase, the three extracts were place in the relative order of inhibition: CSE > WSE > ASE. During the formation of the substrate emulsion, the extract could affect the particle size of TBG droplets, a parameter governing the lipase activity. So the interface was influenced and the rate of hydrolysis reduced because that the lipase-catalyzed hydrolysis of TBG occurred at the interface. Moreover, the increase of inhibition effect was related to increasing concentration of the extracts, which could be attributed to the increase in viscosity which significantly reduced the amount of emulsified triglyceride, increased the droplet size of the emulsions and decreased 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).

4. Conclusion

WSE, CSE and ASE were extracted from *C. edulis* by-product using water, 0.5% oxalate ammonium and dilute HCl solution, respectively, and their thermal characteristics and physiological behaviors were investigated in this paper. WSE and ASE exhibited similar thermal properties of cellulose, as main constituents in the extracts. Different from those of WSE and ASE, the thermal characteristics of CSE demonstrated that it was chiefly composed of cellulose and pectin. The three extracts inhibited both gastric pepsin and lipase enzymatic activities to some extent, and tryptic digestion of β-lactoglobulin as well as lipase hydrolysis of tributyrin in vitro. Therefore, the three extracts could be used in the food industry as additives.

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