Carbohydrate Polymers 101 (2014) 479–483

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Separation of water-soluble polysaccharides from *Cyclocarya paliurus* by ultrafiltration process

Jian-Hua Xie [1], Ming-Yue Shen [1], Shao-Ping Nie, Qiang Zhao, Chang Li, Ming-Yong Xie *

State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, China

ARTICLE INFO

Article history:
Received 17 August 2013
Received in revised form
15 September 2013
Accepted 21 September 2013
Available online 29 September 2013

Keywords:
Cyclocarya paliurus (Batal.) Iljinskaja
Polysaccharide
Ultrafiltration
Membrane
Molecular weight

ABSTRACT

In this study, ultrafiltration membrane process was employed to separate polysaccharides from *Cyclocarya paliurus* (Batal.) Iljinskaja (*C. paliurus*) to simulate industrial production. Meanwhile, the molecular weight distribution of *C. paliurus* polysaccharides was investigated by gel permeation chromatography. Four fractions were obtained and named as CPPS-A, CPPS-B, CPPS-C and CPPS-D, respectively. CPPS-A and CPPS-B contained approximately 69.5% and 12.7% of polysaccharides, whose molecular weight were in the range of 100–300 kDa and 120 kDa, respectively. CPPS-C was comprised of two polysaccharides with average molecular weight of 40 kDa and 15 kDa. Results showed that ultrafiltration resulted in the removal of parts of small molecule weight polysaccharides, the increase of proportion of high molecule weight ones and the obvious improvement of quality of products. Compared with ethanol precipitation and gel permeation chromatography techniques, ultrafiltration showed many advantages, and also provided theoretical support for industrial manufacturing of *C. paliurus* polysaccharides in separation.

1. Introduction

Cyclocarya paliurus (Batal.) Iljinskaja (*C. paliurus*), namely sweet tea tree in China, has been used in Chinese medicine for treatment of hyperglycemia, hyperlipidemia and diabetes mellitus for thousands of years (Kurihara, Asami, Shibata, Fukami, & Tanaka, 2003; Xie, Li, Nie, Wang, & Lee, 2006; Xie et al., 2010a), and was also used as beverages (such as herbal tea) in China (Xie et al., 2006). It has been found that *C. paliurus* contains many potentially bioactive substances, including polysaccharides, proteins, minerals, phenolic compounds, vitamins, saponins, sugars, triterpenoids and amino acids (Xie & Xie, 2008; Xie et al., 2013a, 2013b). As the major active constitute in *C. paliurus* leaves, *C. paliurus* polysaccharides have been shown to contain 23.5% uronic acid and 64.8% neutral sugars (Xie et al., 2010b), which consisted of rhamnose, arabinose, galactose, glucose, mannose and xylose in the molar ratio of 1.00:1.85:3.26:3.12:0.85:0.29 (Xie et al., 2013c). We have reported that the polysaccharides in *C. paliurus* possess a variety of bioactivities, such as antioxidant (Xie et al., 2010b), hypoglycemic (Xie et al., 2006), anticancer (Xie et al., 2013d), antimicrobial (Xie et al., 2012) and immunomodulatory activities (Huang, Nie, Xie, Han, & Xie, 2009).

In recent decades, polysaccharides isolated from plants, animals and fungi have attracted a great deal of attention due to their various biological activities (Nie, Cui, Xie, Phillips, & Phillips, 2013). However, most current studies on polysaccharide put an emphasis on its structure or bioactivities. There have been few reports on membrane separation and purification of polysaccharides (Sheng et al., 2007).

Various methods, such as ethanol precipitation, gel permeation chromatography and ion-exchange chromatography have been employed for separation and purification of polysaccharides. However, these methods possess inherent limitations. The traditional ethanol precipitation used to separate polysaccharides is time-consuming and requires large volumes of organic solvents (Du et al., 2010). The gel filtration chromatography is not only complicated but also expensive (Hong & Choi, 2007). Compared to conventional separation techniques, ultrafiltration membrane process has many advantages, such as low cost, energy saving, high efficiency, and being environmental friendly, having the characteristics of continuous operation at room temperature, and no secondary dissolving-out substances in the separation (Mohammad, Ng, Lim, & Ng, 2012). The ideal separation method should be capable of producing high quantities of polysaccharides and be non-destructive with a shorter separation time (Sheng et al., 2007). Therefore, the use of ultrafiltration process for separation of molecular weight-specific polysaccharides with particular biological activities has

* Corresponding author at: State Key Laboratory of Food Science and Technology, Nanchang University, No. 235 Nanjing East Road, Nanchang 330047, Jiangxi, China. Tel.: +86 791 83969009; fax: +86 791 83969009.
E-mail addresses: jhxie@ncu.edu.cn (J.-H. Xie), xmync@163.com, myxie@ncu.edu.cn (M.-Y. Xie).
[1] These authors contributed equally to this work and are considered as co-first authors.

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.09.075

received increased attention. In recent years, it has been applied in separation and purification of many biomolecules such as proteins (Cheang & Zydney, 2004; Tsou, Kao, Juin Tseng, & Chiang, 2010), antibiotics (Hernández-Campos, Brito-De la Fuente, & Torrestiana-Sánchez, 2010), peptides (Chabeaud et al., 2009; Liu et al., 2013; Segura Campos, Chel Guerrero, & Betancur Ancona, 2010), polyphenols (Nawaz, Shi, Mittal, & Kakudac, 2006; Prodanov et al., 2008) and polysaccharides (Du et al., 2010; Sun, Qi, Xu, Juan, & Zhe, 2011). To our knowledge, the suitability of ultrafiltration membrane for direct separation of *C. paliurus* polysaccharides has not been reported. In this study, ultrafiltration membrane process was successfully employed for the separation of *C. paliurus* polysaccharides and four fractions were obtained.

2. Materials and methods

2.1. Materials and chemicals

The leaves of *C. paliurus* were collected in Xiushui County, Jiangxi Province, China. A voucher specimen was deposited at State Key Laboratory of Food Science and Technology, Nanchang University, China. The leaves were air dried and ground into a fine powder in a mill.

Standard dextrans, namely, T-10, T-40, T-70, T-500 and T-2000 (10, 40, 70, 500 and 2000 kDa) were purchased from Pharmacia Biotech (Uppsala, Sweden). Aqueous solutions were prepared with ultra pure water from a Milli-Q water purification system (Millipore, Bedford, MA, USA). All other chemicals and solvents used were of analytical reagent grade unless otherwise specified.

2.2. Extraction of crude C. paliurus polysaccharides

Extraction of water-soluble *C. paliurus* polysaccharides was carried out by the procedure reported in our previous study (Xie et al., 2011). Briefly, the dried leaves powder (500 g) were extracted with 5000 mL of 80% ethanol for 24 h at room temperature to remove the interference components such as monosaccharide, disaccharide and polyphenol in the samples. Then the pretreated samples were dried at 45 °C for 8 h. The dried residues were extracted with 5000 mL ultra pure water at 80 °C for 2 h. After filtered, the residues were extracted again with 2500 mL ultra pure water at the same temperature for 2 h. Then the extracts were combined and filtered through glass wool and centrifuged at 8400 × *g* for 15 min to separate the supernatant and the residue. The supernatant was kept in a refrigerator for membrane separation.

2.3. Separation of C. paliurus polysaccharides by ultrafiltration

The *C. paliurus* polysaccharides solution was pretreated through a membrane with glass wool to avoid the fouling of the ultrafiltration membranes. The ultrafiltration process was performed on an ultrafiltration system with different membranes. The polysaccharides solution was pumped to the membrane surface (tangential flow) and the filtrate was collected while the retentate was directed back to the recycle tank. All experiments were carried out with a hollow fiber ultrafiltration module (Suntar Membrane Technology Co., Ltd., Xiamen, China) with different nominal molecular weight cut-offs (300 kDa, 100 kDa and 6 kDa) polysulfone hollow fiber membranes (effective membrane area: 0.15 m^2). The polysaccharide solutions were pumped by a peristaltic pump (PP-05, Chenghe Co., Ltd., Changzhou, China). The schematic of ultrafiltration set-up is shown in Fig. 1.

The *C. paliurus* polysaccharide solution prepared by centrifugation (8400 × *g* for 15 min) was passed through the membrane starting with the largest membrane cartridge (300 kDa). The retentate and permeate were collected separately, and the retentate

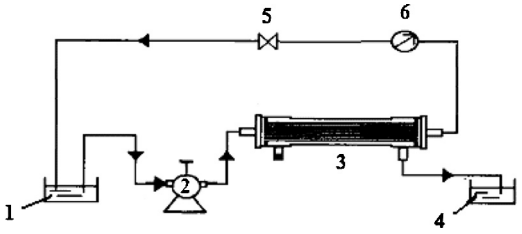

Fig. 1. Experimental set-up of ultrafiltration with hollow fiber. (1) sample liquid pool; (2) peristaltic pump; (3) ultrafiltration module; (4) permeated liquid pool; (5) regulating valve; (6) septum manometer.

was recirculated into the feed until maximum permeate yield was reached, as indicated by a decreased permeate flow rate. Permeate from the 300 kDa membrane was then filtered through the 100 kDa membrane with recirculation until maximum permeate yield was reached, and the 100 kDa permeate was then processed with the 6 kDa membrane as mentioned above (Fig. 2).

Four fractions (CPPS-A, CPPS-B, CPPS-C and CPPS-D) obtained from *C. paliurus* polysaccharide solution were concentrated to small volumes and also precipitated with four volumes of anhydrous ethanol for 12 h at 4 °C. The four precipitates were dissolved in ultra pure water, and freeze-dried respectively.

2.4. Molecular size distribution of C. paliurus polysaccharides

The molecular weight of the polysaccharide fractions was determined by high-performance gel permeation chromatography (HPGPC) with a Waters HPLC apparatus (UK6 injector and 515 HPLC pump, Waters, Milford, MA, USA) equipped with an Ultrahydrogel TM-500 column (300 × 7.8 mm), a Waters 2410 refractive index detector (Waters Corp., USA) and a Waters 2487 UV detector connected in series with a Millennium32 workstation.

The elution was carried out with Milli-Q water at a flow rate of 0.6 mL/min. All sample solutions were filtered through a 0.45 μm membrane filter prior to analysis. The injection volume of standards and samples was 20 μL, and the running time was 30 min. Retention times from HPGPC were recorded and time variations were dependent on the average molecular weight of the dextran standards. Glucose and dextran standards with different molecular weights (10, 40, 70, 500 and 2000 kDa) were prepared using the same solvent as the mobile phase. A standard curve with the retention time (t) plotted against the logarithm of their respective molecular weights (Mw). The equation of the standard curve was: $\log M_w = 8.7928 - 0.3062t$ (where M_w represented the molecular weight, while t represented retention time) with a correlation coefficient of 0.991 (Xie et al., 2010b).

2.5. Determination of polysaccharides content

The polysaccharide content of the four fractions was determined according to the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) with some modifications. Briefly, 2 mL of diluted solution containing polysaccharides, 1.0 mL of 5% (w/w) phenol was added in the tubes and mixed up by shaking quickly. Then 5 mL of sulfuric acid was added and mixed, and boiled in the boiling water for 20 min, and the tubes were cooled down to room temperature by tap water, and finally 2 mL ultra pure water was added and mixed. The absorbance of the solution was measured at 488 nm with an ultraviolet-visible spectrophotometer (TU-1900, Pgenenal, Beijing, China) against the same mixture, without adding the sample as a blank. The calibration curve of glucose ($y = 5.441x + 0.025$, where y is the absorbance value of sample, x is the polysaccharide concentration, $R^2 = 0.995$) is shown in Fig. 3.

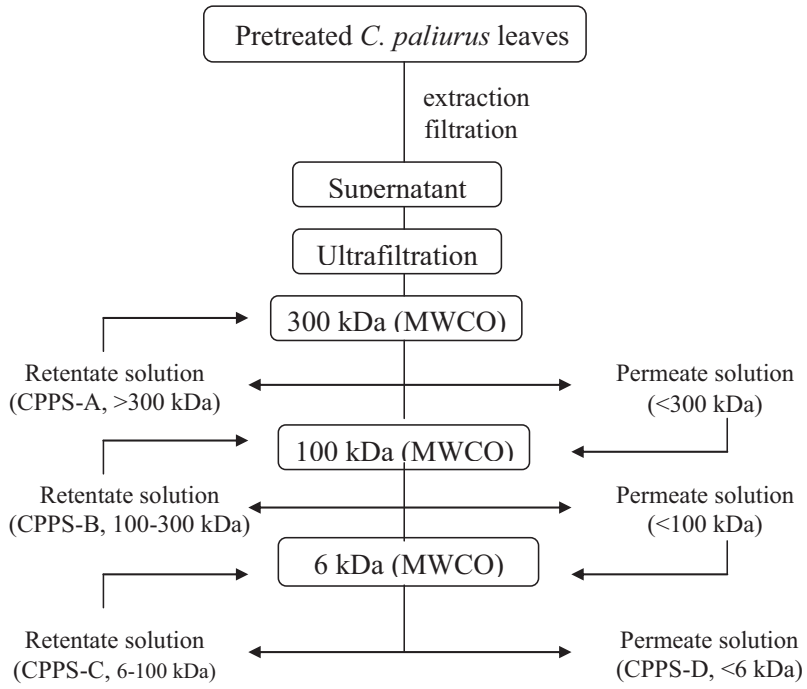

Fig. 2. Scheme of fractionation of polysaccharides with different molecular weights from *C. paliurus* leaves.

2.6. Statistical analysis

Data were expressed as means ± standard deviations (S. D.) and the results were taken from at least three independent experiments performed in triplicate. The statistical significance was estimated using a Student's *t*-test and the differences were considered as statistically significant with $P < 0.05$.

3. Results and discussion

The ultrafiltration membranes of different molecular weight cut-offs (300 kDa, 100 kDa and 6 kDa) were used successively to identify the main fractions of the *C. paliurus* polysaccharides. Fig. 4 presents the fractions of the polysaccharides that were not filtered by the three membranes. There are four different molecular weight groups, namely, CPPS-A (>300 kDa), CPPS-B (100–300 kDa), CPPS-C (6–100 kDa) and CPPS-D (<6 kDa). Clearly, the fraction cut off by the 300 kDa membrane (CPPS-A) had the greatest yield, of 69.5% by mass percentage, significantly higher than any other fractions ($P < 0.05$). The percentage of the polysaccharides of CPPS-B (100–300 kDa) and CPPS-D (<6 kDa) was 12.7% and 10.1%, respectively, and the lowest fraction was CPPS-C (6–100 kDa), which only accounts for 6.7% of the resulting polysaccharides (Fig. 4).

In order to explore the molecular weight distribution of the *C. paliurus* polysaccharides, the feed solution was in turn filtrated through the three membranes and the polysaccharides that were not filtered were analyzed by HPGPC. From HPGPC analysis, the molecular weight distribution of the polysaccharides was analyzed by using the response (mV, obtained from the refractive index detector) data of HPLC with retention time. The molecular weight

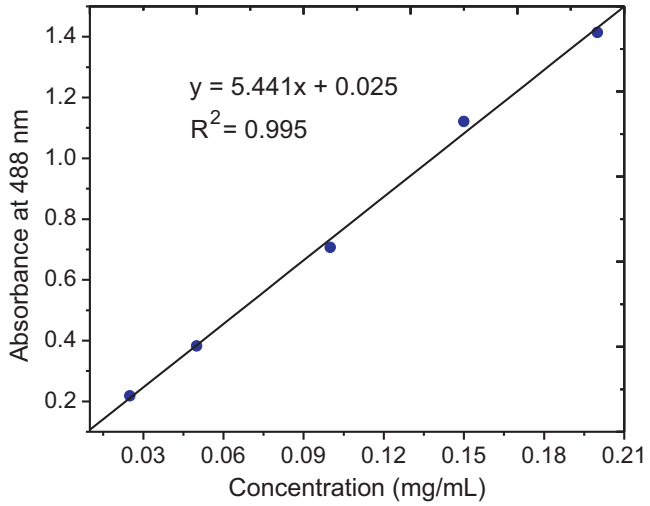

Fig. 3. Calibration curve of glucose.

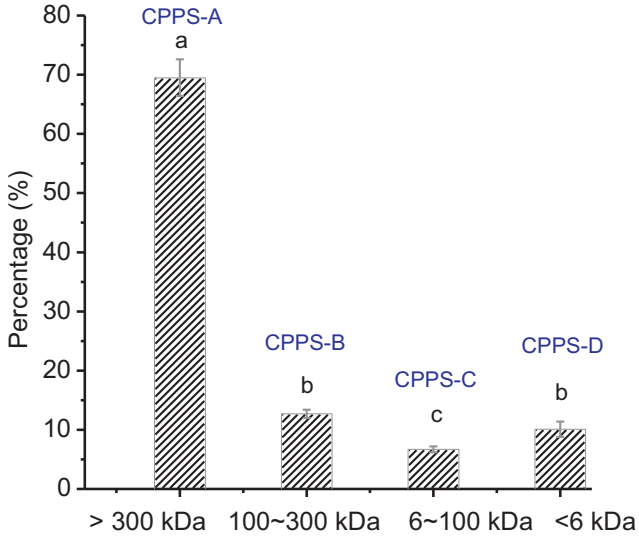

Fig. 4. Main fraction of the *C. paliurus* polysaccharides obtained by ultrafiltration membranes (relative percentage of each molecular weight class). (a–c) Different superscript letters indicate statistical difference ($P < 0.05$). Data are the mean values of three replicates.

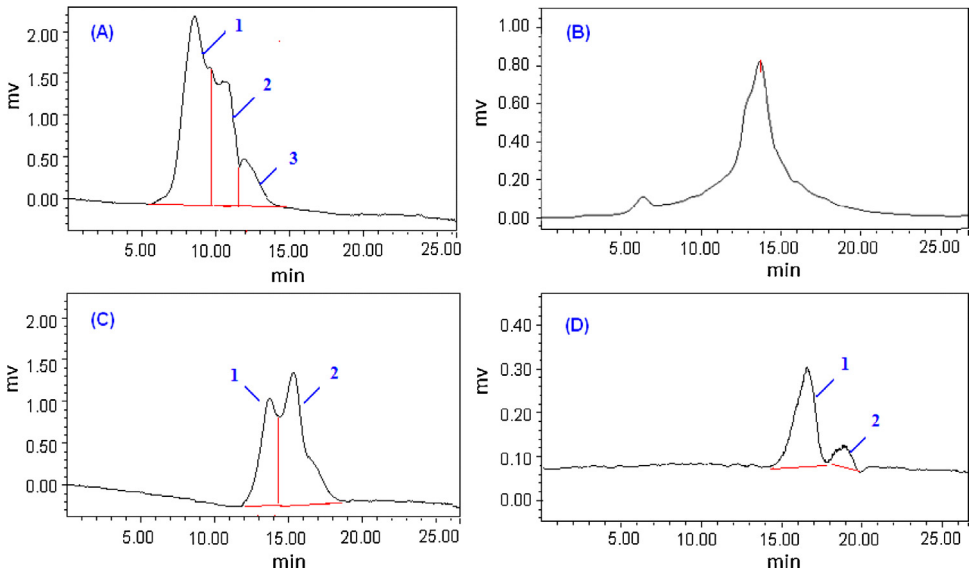

Fig. 5. HPGPC chromatogram of polysaccharides rejected by ultrafiltration membranes of different molecular weight cut-offs. (A) CPPS-A, (B) CPPS-B, (C) CPPS-C, and (D) CPPS-D.

distribution of *C. paliurus* polysaccharides were determined according to the calibration curve ($\log M_w = 8.7928 - 0.3062t$) (Fig. 5).

CPPS-A was eluted as three main fractions, namely, CPPS-A1, CPPS-A2 and CPPS-A3, with the molecular weights of 1100 kDa, 400 kDa and 130 kDa, respectively. CPPS-B was eluted as a single peak, whose average molecular weight was estimated to be 120 kDa. CPPS-C consisted of two polysaccharides with average molecular weights of 40 kDa and 15 kDa. Moreover, CPPS-D was eluted as a small peak followed by a main peak, indicating that CPPS-D is comprised of a polysaccharide with average molecular weight of 5 kDa and a monosaccharide. These results suggested that some polysaccharides of molecular weights below 300 kDa might be lost if filtrated with molecular weight cut-off 300 kDa.

Crude water-soluble polysaccharides of *C. paliurus* were extracted with hot water. Although the ethanol soluble components were removed by pretreatment, the residual constituents with low molecular weights including monosaccharides, oligosaccharides and phenolics remained in the water extracts. In order to obtain the desired production efficiency of membrane separation, cleaning of ultrafiltration membrane is required. In this experiment, *C. paliurus* polysaccharide solutions were filtrated through a membrane with glass wool before ultrafiltration to avoid the fouling of the ultrafiltration membranes.

Since *C. paliurus* polysaccharide solution is the large molecular material with weak acidity, the chemically–physically combined method was adopted to clean the ultrafiltration membrane. The cleaning of the fouled membrane at the end of each run was performed according to the manufacturer's instructions. Briefly, clean water was first used to wash away the big molecular matters on membrane surface, and then, NaOH solution (pH 8.5) was used to neutralize the *C. paliurus* polysaccharides solution with weak acidity attached to membrane surface. Furthermore, clean water was used to wash away base solution completely so that the recovery of membrane flux was excellent. And at last, a cleaning step using aqueous solution of NaClO (300 mg/L) was carried out to ensure sanitation and cleaning. Although chemical cleaning can be very effective to remove fouling as compared to other techniques, it may severely damage the membrane materials and thus reduce membrane lifespan (Mohammad et al., 2012). Previous studies on the use of enzymatic cleaners have been reported. For example, Petrus, Li, Chen, and Norazmanet (2008) used enzymatic cleaning agent to clean the ultrafiltration membranes fouled by protein solution.

Ultrafiltration was compared with the ethanol precipitation and gel permeation chromatography techniques for the separation of polysaccharides from *C. paliurus*. The results were given in Table 1. As for the treatment time, membrane separation method needed less time than the gel permeation chromatography method, while the time of the ethanol precipitation reached even more than 12 h. In terms of the amount of reagents used, ultrafiltration showed the best method for the separation of polysaccharides, which could avoid the loss of bioactive substances during treatment. Ethanol precipitation seems to be a simple method for obtaining polysaccharide, but this method consumes plenty of organic solvent, and the product purity is very low. In order to obtain the product with high purity, a further purification process must be performed (Tan, Li, Xu, & Xing, 2012). In general, gel permeation chromatography is frequently used for extraction procedures. The drawbacks of this procedure include a need of large amount of energy, high cost and complex operations and long time, which restricts its large scale application (Tan et al., 2012). However, the membrane separation method had far greater product purity than the traditional ethanol precipitation method, indicating that membrane separation method could remove most of the impurities from raw solution, enhance the product purity, and have the function of preliminary purification. This is in agreement with ultrafiltration for the separation of polysaccharides from *Fructus lycii* (Fan, Yu, Zhang, & Bao 2011) and rapeseed (Sun et al., 2011). Lang, Ma, Zhao, and Zeng (2013) have used the ultrafiltration to purify and concentrate polysaccharides from Rhizomes of *Stachys floridana* to obtain better quality of polysaccharides than alcohol precipitation.

The viscosity of polysaccharide can influence the ultrafiltration separation. If the viscosity of polysaccharides solution is very high,

Table 1
Comparison of ultrafiltration and other common laboratory separation techniques.

	Ethanol precipitation	Gel permeation chromatography	Ultrafiltration
Processing capacity (mL)	Large	0.5–5	500–1000
Duration (h)	12–24	0.5–4	1–6
Amount of reagents required	Large	Small	Very small
Product purity	Low	High	Moderate
Need of monitor	No	Yes	No

the membrane is easily fouled. However, the viscosity of *C. paliurus* polysaccharides solution is very low. Our previous study (Xie et al., 2013d) showed the viscosity was approximately 3.1 mPa s at a concentration of 1 mg/mL. Furthermore, the ultrafiltration is a non-thermal process, does not require chemical compounds and is economically more viable than other treatments. At the same time, membrane separation method had the characteristics of mild operating conditions and simple operation, and had great prospects for industrial application.

4. Conclusion

In conclusion, ultrafiltration membrane separation technology is a separation technology with advantages including high-efficiency, low cost, energy saving, and being environmental friendly. In this study, four polysaccharide fractions (CPPS-A, CPPS-B, CPPS-C and CPPS-D) with different molecular weights were successively isolated by ultrafiltration method from crude polysaccharide extracted from *C. paliurus* leaves. Therefore, ultrafiltration is a good alternative approach for the separation of *C. paliurus* polysaccharides, and it may be also used for the separation of other biologically active polysaccharides from other plant materials.

Acknowledgements

Authors are grateful for financial supports from National Natural Science Foundation of China (No. 31201297), the Key Program of Natural Science Foundation of China (No. 31130041), the Natural Science Foundation for Young Scientists of Jiangxi Province, China (No. 20122BAB214004), the National Key Technology R & D Program of China (No. 2012BAD33B06) and the Project of State Key Laboratory of Food Science and Technology, China (SKLF -QN-201104).

References

Chabeaud, A., Vandanjon, L., Bourseau, P., Jaouen, P., Chaplain-Derouiniot, M., & Guerard, F. (2009). Performances of ultrafiltration membranes for fractionating a fish protein hydrolysate: Application to the refining of bioactive peptidic fractions. *Separation and Purification Technology*, *66*(3), 463–471.

Cheang, B., & Zydney, A. L. (2004). A two-stage ultrafiltration process for fractionation of whey protein isolate. *Journal of Membrane Science*, *231*(1-2), 159–167.

Du, X. J., Zhang, J. S., Yang, Y., Tang, Q. J., Wei, J., & Pan, Y. J. (2010). Purification, chemical modification and immunostimulating activity of polysaccharides from *Tremella aurantialba* fruit bodies. *Journal of Zhejiang University-Science B*, *11*(6), 437–442.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*(3), 350–356.

Fan, Y. J., Yu, F. F., Zhang, P., & Bao, Q. G. (2011). Separation technology of polysaccharides from *Fructus lycii* by ultrafiltration method. *Medicinal Plant*, *2*(8), 61–65.

Hernández-Campos, F., Brito-De la Fuente, E., & Torrestiana-Sánchez, B. (2010). Purification of egg yolk immunoglobulin (IgY) by ultrafiltration: Effect of pH, ionic strength, and membrane properties. *Journal of Agricultural and Food Chemistry*, *58*(1), 187–193.

Huang, D. F., Nie, S. P., Xie, J. H., Han, C., & Xie, M. Y. (2009). Effect of *Cyclocarya paliurus* (Batal) Iljinskaja polysaccharides on the surface molecule expression level of dendritic cells. *Natural Product Research and Development*, *21*(5), 771–775.

Hong, J. H., & Choi, Y. H. (2007). Physico-chemical properties of protein-bound polysaccharide from *Agaricus blazei Murill* prepared by ultrafiltration and spray drying process. *International Journal of Food Science and Technology*, *42*(1), 1–8.

Kurihara, H., Asami, S., Shibata, H., Fukami, H., & Tanaka, T. (2003). Hypolipemic effect of *Cyclocarya paliurus* (Batal) Iljinskaja in lipid-loaded mice. *Biological and Pharmaceutical Bulletin*, *26*(3), 383–385.

Lang, C. Y., Ma, L. P., Zhao, K., & Zeng, X. X. (2013). Orthogonal array optimization of ultrafiltration concentration of polysaccharides from rhizomes of *Stachys floridana* Schuttl. ex Benth. *Food Science*, *34*(4), 51–54.

Liu, J. H., Huang, Y. S., Tian, Y. G., Nie, S. P., Xie, J. H., Wang, Y., et al. (2013). Purification and identification of novel antioxidative peptide released from Black-bone silky fowl (*Gallus gallus domesticus Brisson*). *European Food Research and Technology*, *237*(2), 163–253.

Mohammad, A. W., Ng, C. Y., Lim, Y. P., & Ng, G. H. (2012). Ultrafiltration in food processing industry: Review on application, membrane fouling, and fouling control. *Food and Bioprocess Technology*, *5*(4), 1143–1156.

Nawaz, H., Shi, J., Mittal, G. S., & Kakudac, Y. (2006). Extraction of polyphenols from grape seeds and concentration by ultrafiltration. *Separation and Purification Technology*, *48*(2), 176–181.

Nie, S. P., Cui, S. W., Xie, M. Y., Phillips, A. O., & Phillips, A. G. (2013). Bioactive polysaccharides from *Cordyceps sinensis*: Isolation, structure features and bioactivities. *Bioactive Carbohydrates and Dietary Fibre*, *1*(1), 38–52.

Petrus, H. B., Li, H., Chen, V., & Norazman, N. (2008). Enzymatic cleaning of ultrafiltration membranes fouled by protein mixture solutions. *Journal of Membrane Science*, *325*(2), 783–792.

Prodanov, M., Garrido, I., Vacas, V., Lebrón-Aguilar, R., Dueñas, M., Gómez-Cordovés, C., et al. (2008). Ultrafiltration as alternative purification procedure for the characterization of low and high molecular-mass phenolics from almond skins. *Analytica Chimica Acta*, *609*(2), 241–251.

Segura Campos, M. R., Chel Guerrero, L. A., & Betancur Ancona, D. A. (2010). Angiotensin-I converting enzyme inhibitory and antioxidant activities of peptide fractions extracted by ultrafiltration of cowpea *Vigna unguiculata* hydrolysates. *Journal of the Science of Food and Agriculture*, *90*(14), 2512–2518.

Sheng, J. C., Yu, F., Xin, Z. H., Zhao, L. Y., Zhu, X. J., & Hu, Q. H. (2007). Preparation, identification and their antitumor activities in vitro of polysaccharides from *Chlorella pyrenoidosa*. *Food Chemistry*, *105*(2), 533–539.

Sun, H. J., Qi, D., Xu, Y., Juan, S., & Zhe, C. (2011). Fractionation of polysaccharides from rapeseed by ultrafiltration: Effect of molecular pore size and operation conditions on the membrane performance. *Separation and Purification Technology*, *80*(3), 670–676.

Tan, Z. J., Li, F. F., Xu, X. L., & Xing, J. M. (2012). Simultaneous extraction and purification of aloe polysaccharides and proteins using ionic liquid based aqueous two-phase system coupled with dialysis membrane. *Desalination*, *286*, 389–393.

Tsou, M. J., Kao, F., Juin Tseng, C. K., & Chiang, W. D. (2010). Enhancing the anti-adipogenic activity of soy protein by limited hydrolysis with Flavourzyme and ultrafiltration. *Food Chemistry*, *122*(1), 243–248.

Xie, J. H., Shen, M. Y., Nie, S. P., Li, C., & Xie, M. Y. (2011). Decolorization of polysaccharides solution from *Cyclocarya paliurus* (Batal.) Iljinskaja using ultrasound/H_2O_2 process. *Carbohydrate Polymers*, *84*(1), 255–261.

Xie, J. H., Shen, M. Y., Nie, S. P., Liu, X., Yin, J. Y., Huang, D. F., et al. (2013). Simultaneous analysis of 18 mineral elements in *Cyclocarya paliurus* polysaccharide by ICP-AES. *Carbohydrate Polymers*, *94*(1), 216–220.

Xie, J. H., Shen, M. Y., Nie, S. P., Li, C., Gong, D. M., & Xie, M. Y. (2013). Proximate, amino acid and fatty acid composition in the leaves of *Cyclocarya paliurus* (Batal.) Iljinskaja. *Journal of Food Agriculture and Environments*, *11*(3-4), 132–136.

Xie, J. H., Shen, M. Y., Nie, S. P., Liu, X., Zhang, H., & Xie, M. Y. (2013). Analysis of monosaccharide composition of *Cyclocarya paliurus* polysaccharide with anion exchange chromatography. *Carbohydrate Polymers*, *98*(1), 976–981.

Xie, J. H., Shen, M. Y., Xie, M. Y., Nie, S. P., Chen, Y., Li, C., et al. (2012). Ultrasonic-assisted extraction, antimicrobial and antioxidant activity of *Cyclocarya paliurus* (Batal.) Iljinskaja polysaccharides. *Carbohydrate Polymers*, *89*(1), 177–184.

Xie, J. H., Liu, X., Shen, M. Y., Nie, S. P., Zhang, H., Li, C., et al. (2013). Purification, physicochemical characterisation and anticancer activity of a polysaccharide from *Cyclocarya paliurus* leaves. *Food Chemistry*, *136*(3-4), 1453–1460.

Xie, J. H., Xie, M. Y., Nie, S. P., Shen, M. Y., Wang, Y. X., & Li, C. (2010). Isolation, chemical composition and antioxidant activities of a water-soluble polysaccharide from *Cyclocarya paliurus* (Batal.) Iljinskaja. *Food Chemistry*, *119*(4), 1626–1632.

Xie, J. H., Xie, M. Y., Shen, M. Y., Nie, S. P., Wang, Y. X., & Li, C. (2010). Optimisation of microwave-assisted extraction of polysaccharides from *Cyclocarya paliurus* (Batal.) Iljinskaja using response surface methodology. *Journal of the Science of Food and Agriculture*, *90*(8), 1353–1360.

Xie, M. Y., Li, L., Nie, S. P., Wang, X. R., & Lee, F. S. C. (2006). Determination of speciation of elements related to blood sugar in bioactive extracts from *Cyclocarya paliurus* leaves by FIA-ICP-MS. *European Food Research and Technology*, *223*(2), 202–209.

Xie, M. Y., & Xie, J. H. (2008). Review about the research on *Cyclocarya paliurus* (Batal.) Iljinskaja. *Journal of Food Science and Biotechnology*, *27*(1), 113–121.