

## Research Article

### Decoloration and Deproteinization Technology of the Alkali-Extractable Polysaccharide from Perilla Seed Meal by Adsorption Resin

Jianfei Zhu and Ling Hu

Chongqing Key Laboratory of Catalysis and Functional Organic Molecules, School of Environmental and Biological Engineering, Chongqing Technology and Business University, Chongqing 400067, People's Republic of China

**Abstract:** To adopt the deprotein and decoloration technology is in order to obtain the purified polysaccharide for further analysis of monosaccharide composition and structural characteristics. Deprotein and decoloration effects of adsorption resins DM301, DM130, NKA-9, NKA-II, H103, ADS-17, D101 and X-5 for alkali extraction solution of perilla meal polysaccharide were compared. In static adsorption experiment research basis, some relatively good adsorption resins were selected to fulfill the dynamic adsorption experiments. The results showed that D101 resin had the best capacity of adsorbing proteins and pigments, indicating it had the best deproteinization and decoloration effects. The recovery rate of polysaccharide was highest also. So D101 resin is the most appropriate resin for the preliminary purification of perilla meal polysaccharide.

**Keywords:** Adsorption resin, perilla meal, polysaccharide, purification

## INTRODUCTION

Perilla seed meal originated from the process of perilla seed oil extraction, is an important agricultural by product, owing to its enriched content of bioactive compounds. The development and utilization of these bioactive compounds have received considerable attention in recent years to increase the profitability of perilla as an agricultural commodity (Zhu and Fu, 2012; Yamamoto and Ogawa, 2002; Longvah and Deosthale, 1998; Shin and Kim, 1994; Tong and Liu, 2008; Zhu *et al.*, 2011). Perilla seeds represent a source of dietary protein and fat in China, very little is known about the separation and purification technology of perilla seeds polysaccharide.

Till now, ethanol, H<sub>2</sub>O<sub>2</sub>, activated carbon and Sevege reagent have been widely used for the decoloration or deproteinization of crude polysaccharide (Li *et al.*, 2005; West and Reed-Hamer, 1993; Wang *et al.*, 2010). These extraction, precipitation, or chemical approaches are often time and volume consuming, labor intensive and difficult to automate and they may cause partial hydrolysis of polysaccharide, resulting in variable bioactivities. Adsorption resins have been widely used in the separation and purification of targeted component or in the removal of the impurities from the crude samples (Liu *et al.*, 2010a; Cheison *et al.*, 2007; Ma *et al.*, 2009; Tang *et al.*, 2001), while little information was

available about their application in simultaneous decoloration and deproteinization of crude polysaccharide. Toward this objective, experiments have been carried out in this study to evaluate the purification effect of crude polysaccharide on eight Adsorption resins (DM301, DM130, NKA-9, NKA-II, H103, ADS-17, D101 and X-5) through static tests. Then, the adsorption efficiency of the selected resin was improved through dynamic adsorption and desorption tests.

## MATERIALS AND METHODS

**Adsorbents, chemicals and standards:** Eight Adsorption resins coded DM301, DM130, NKA-9, NKA-II, H103, ADS-17, D101 and X-5 were provided by Anhui Sanxing Resin Technology Co., Ltd (Anhui, China). Their physical and chemical properties are listed in Table 1. Before the adsorption experiment, each weighed resin (10 g) were soaked in 200 mL of 95% ethanol and washed thoroughly with 1 L of deionized water and the pretreated with 200 mL of HCl (1 M) and 200 mL of NaOH (1 M) solutions sequentially to remove the monomers and porogenic diluents trapped inside the pores. Subsequently, they were washed thoroughly with deionized water until neutral pH. Perilla seed meal was provided by Chengdu Suma biotechnology Co., Ltd.

**Corresponding Author:** Jianfei Zhu, Chongqing Key Laboratory of Catalysis and Functional Organic Molecules, School of Environmental and Biological Engineering, Chongqing Technology and Business University, Chongqing 400067, People's Republic of China, Tel./fax: +86 23-62769672

This work is licensed under a Creative Commons Attribution 4.0 International License (URL: <http://creativecommons.org/licenses/by/4.0/>).

Table 1: Physical and chemical properties of the adsorption resins

	Structure	Polarity	Appearance	Particle diameter (mm)	Surface area (m <sup>2</sup> /g)	Average pore diameter (nm)
DM301	Cross-linked polystyrene	Moderate polar	Milk white	0.3-1.25	330-380	13-17
DM130	Cross-linked polystyrene	Weak polar	Milk white	0.3-1.25	500-550	9-10
NKA-9	Cross-linked polystyrene	Polar	Milk white	0.3-1.25	250-290	15.5-16.5
NKA-II	Cross-linked polystyrene	Polar	Red-brown	0.3-1.25	160-200	14.5-15.5
H103	Cross-linked polystyrene	Polar	Brown	0.3-1.25	900-1100	8.4-9.4
ADS-17	Styrene-divinyl benzene copolymers	Moderate polar	Milk white	0.4-1.0	90-150	25-30
D101	Cross-linked polystyrene	Non-polar	Milk white	0.3-1.25	500-550	9-10
X-5	Cross-linked polystyrene	Weak polar	Milk white	0.3-1.25	500-600	29-30

**Preparation of the alkali extraction solution of polysaccharide:** Defatted perilla seed meal was extracted twice with 80% (v/v) EtOH (1 g/20 mL) at 80°C for 2 h to remove most of the phenolic compounds and oligosaccharides. The pretreated rapeseed meal was then air-dried, ground and extracted with 5 mol/L NaOH (1 g/20 mL) and heated at 80°C for 2 h. Alkali-insoluble material was removed by filtration with the Buchner funnel and the filtrate was acidified to pH 5 with 4 mol/L HCl. The precipitation was allowed to take place for 5 h. The mixture was then centrifuged (4000 g, 10 min), the pellet was discarded and the supernatant was the alkali-soluble polysaccharide solution.

**Measurement of decolorization ratio:** Sample solution was adjusted to pH 7.0 with 1 M HCl and NaOH and then centrifuged at 5000 rpm for 20 min. Subsequently, the absorbance of the resultant supernatant was measured at 420 nm on a UV-1102 spectrophotometer (Tianmei, Shanghai, China). The following equation was used to quantify the decolorization ratio (Li *et al.*, 2013):

$$\text{Decolorization ratio (\%)} = (A_1 - A_2) / A_1 \times 100 \quad (1)$$

where,  $A_1$  and  $A_2$  were the absorbance of the samples at 420 nm before and after adsorption by microspheres, respectively.

**Measurement of protein concentration and polysaccharide recovery:** The concentration of protein was determined according to the method of Bradford (1976) using bovine serum albumin as standard. The following equation was used to quantify the deproteinization ratio:

$$\text{Deproteinization ratio (\%)} = (C_1 - C_2) / C_1 \times 100 \quad (2)$$

where,  $C_1$  and  $C_2$  were the concentrations of protein (mg/L) in the solutions before and after adsorption by adsorption resins, respectively.

**Measurement of polysaccharide concentration and recovery rate of polysaccharide:** The concentration of polysaccharide was measured by the phenol-sulfuric acid method using glucose as standard (Masuko *et al.*, 2005):

$$\text{Recovery rate of polysaccharide (\%)} = C_4 / C_3 \times 100 \quad (3)$$

where,  $C_3$  and  $C_4$  were the concentrations of polysaccharide (mg/L) in the solutions before and after adsorption by adsorption resins, respectively.

**Test for the selection of the resins:** Static adsorption test was performed to evaluate the adsorption and desorption ratios for preliminary screening of the most efficient resin. In the adsorption test, 10 g of resin (dry weight basis) was introduced into a 250 mL airtight conical flask with airtight conical flask with 100 mL of polysaccharide solution (The concentrations of polysaccharide and protein were 0.72 and 0.42 mg/mL, respectively and absorbance at 420 nm was 0.66). The flasks were shaken (150 rpm) at 20°C in a water-bath shaker for 12 h. After the resin was separated from the sample solution by filtration, the decoloration, deproteinization and polysaccharide recovery ratios of the resin were measured. The preliminary selection of the resins was evaluated by their decoloration, deproteinization and polysaccharide recovery ratios towards the polysaccharide solution.

The most suitable resin was further selected from the preliminary selected resins by dynamic adsorption tests, which were carried out as follows: 100 mL of the polysaccharide solution as described above was loaded continuously at a constant flow rate to a glass column (2.4×25 cm) wet-packed with the selected resin. The Bed Volume (BV) of the resin was 100 mL. The decoloration and deproteinization ratios of the effluents (10 mL/tube collected) were monitored.

**Comparison test of the adsorption efficiency by D101 resin in different conditions:** The adsorption time of decoloration and deproteinization efficiencies of the selected resin were investigated by static adsorption tests and the effects of flow rate on decoloration and deproteinization efficiencies of the selected resin were investigated by dynamic adsorption tests.

**Statistical analysis:** The data were presented as mean± Standard Deviation (SD) and evaluated by one-way Analysis of Variance (ANOVA) followed by the

Duncan's multiple-range tests. Difference was considered to be statistically significant if  $p < 0.05$ . All statistical analyses were carried out by SPSS for Windows, Version 11.5 (SPSS, Chicago, IL).

## RESULTS AND DISCUSSION

### Comparison of adsorption tests of different resins:

As shown in Fig. 1, X-5, D101, DM301 and DM130 resins exhibited higher adsorption ratios toward pigment and protein than those of the other four resins, as well as the recovery ratios of polysaccharide. They had no significant differences in decoloration ratios except for a much lower value on NKA-II and H103 resins. They had no significant differences in deprotein ratios except for a much lower value on H103 and ADS-17 resins. They had no significant differences in deprotein ratios except for a much lower value on H103 and ADS-17 resins. X-5, D101, DM301 and DM130 resins were chosen for further study. Figure 2 showed that D101 exhibited higher adsorption ratios toward pigment and protein and recovery ratios of polysaccharide than those of the other three resins in dynamic adsorption tests. The values of that of D101 were 77.14, 78.73 and 79.56%, respectively separately. Therefore, D101 resin was the best one among the eight resins for the purification of alkali extraction solution of polysaccharide from perilla seed meal.

### Effect of adsorption time on the decoloration and deproteinization ratios of D101 resin:

To investigate the effect of adsorption time on the decoloration and deproteinization ratios of D101 resin, the polysaccharide solution were shaken at 35°C. As shown in Fig. 3, the decoloration and deproteinization ratios increased rapidly in the first 30 min and reached a plateau at 90 min. In addition, the polysaccharide recovery ratio decreased with the increase of adsorption time and also reached the plateau at 90 min. These results indicated that the adsorption property of D101 resin reached the equilibrium at around 90 min. It has been suggested that the initial fast adsorption process of the resin in 30 min was due to the high diffusivity of solute molecules into micropores of the resin and the slow adsorption process of the resin after 30 min was due to the high intraparticle mass transfer resistance within the resin (Liu *et al.*, 2010b).

### Effect of flow rate on the decoloration and deproteinization ratios of D101 resin:

When the adsorption process reaches the break point, the adsorption capacity of the resin will decrease or even disappear and the solutes will leak from the resin. It is necessary to investigate into the processing volume and the flow rate of sample solution. To investigate the effect of flow rate on the decoloration and

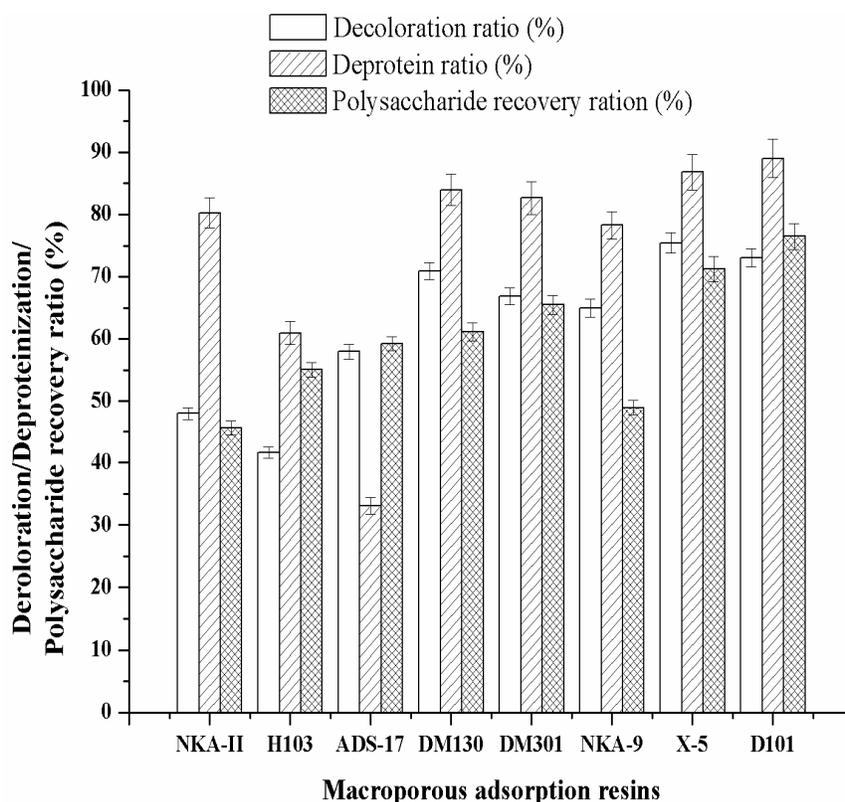


Fig. 1: Decoloration, deproteinization and polysaccharide recovery ratios of different adsorption resins in static adsorption tests. Data are presented as means±SD of triplicates

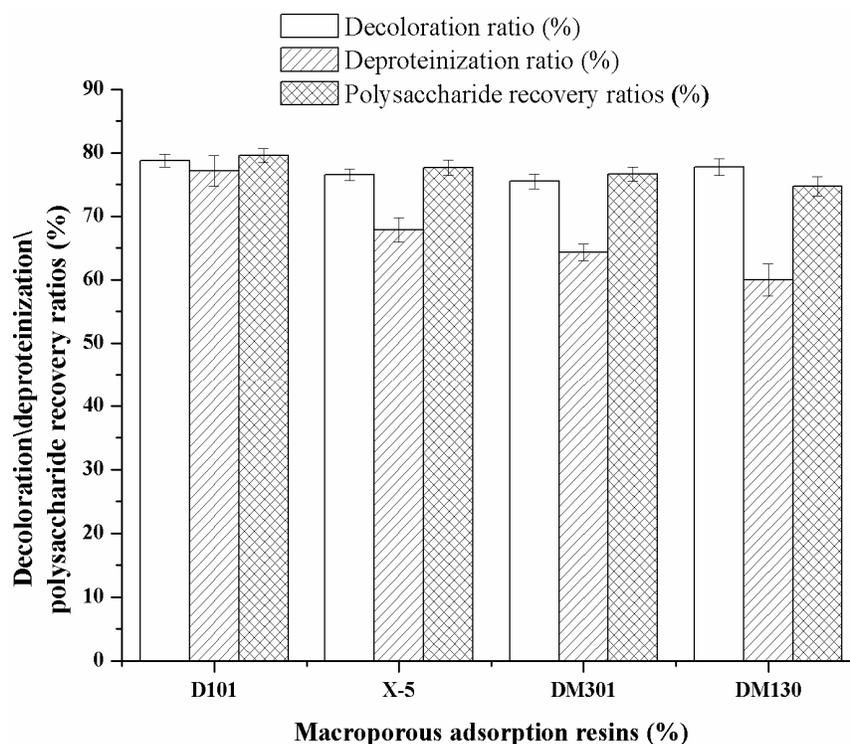


Fig. 2: Decoloration, deproteinization and polysaccharide recovery ratios of different adsorption resins in dynamic adsorption tests. Data are presented as means±SD of triplicates

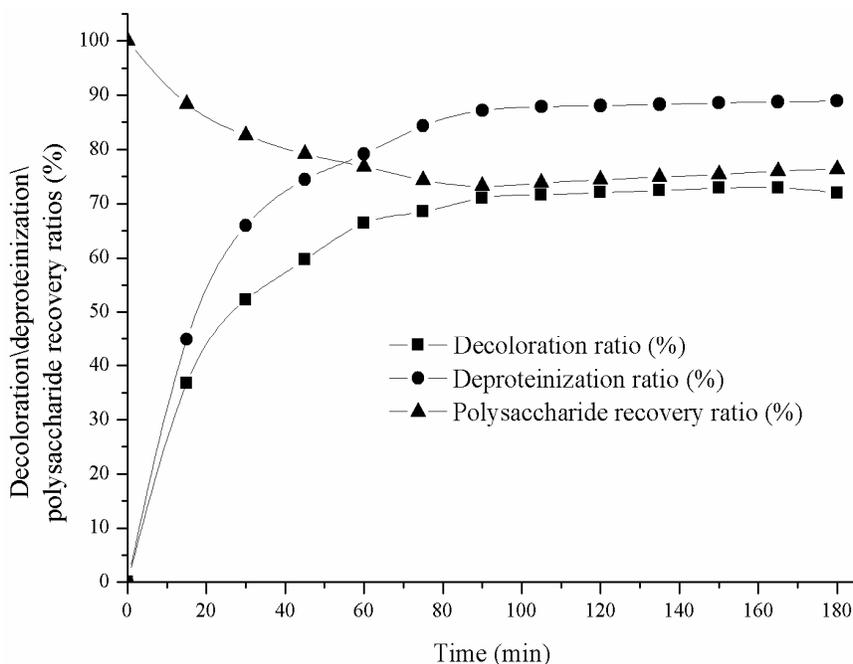


Fig. 3: Effect of adsorption time on decoloration, deproteinization and polysaccharide recovery ratios of D101 resin. Data are presented as means of triplicates

deproteinization ratios, 100 mL of polysaccharide solution was loaded at different flow rate (0.5, 1, 2 and 4 BV/h) to a glass column (2.4×25 cm) wet-packed with 100 mL of D101 resin. As shown in Fig. 4, both of

the decoloration and deproteinization ratios decrease gradually with the increase of the volume of sample solution, as well as the increase of the flow rate of sample solution.

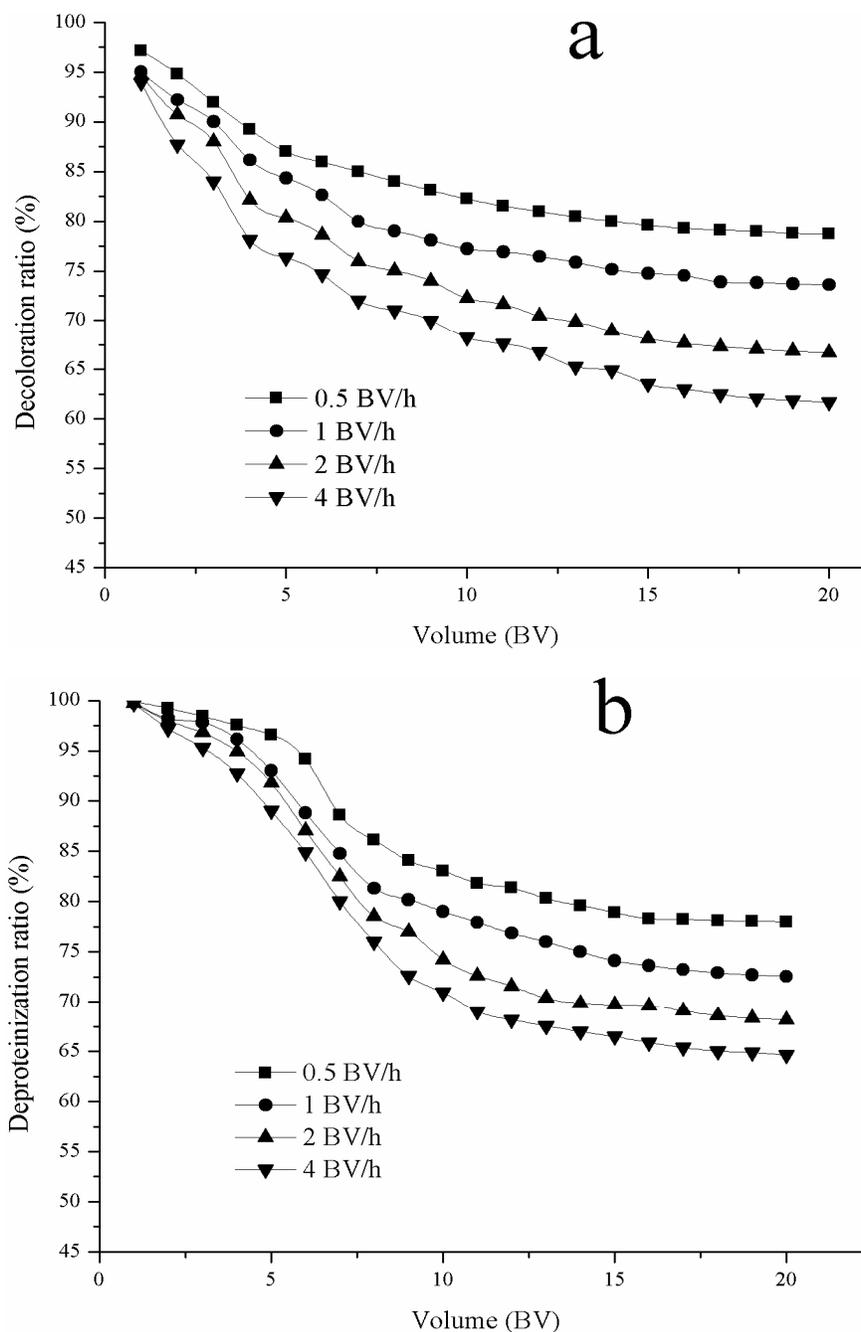


Fig. 4: Effect of flow rate on decoloration; (a) and deproteinization; (b) ratios of D101 resin

### CONCLUSION

Deprotein and decoloration technology is essential in the process of polysaccharide purification. Among the eight adsorption resins including DM301, DM130, NKA-9, NKA-II, H103, ADS-17 and D101, D101 had the best deproteinization and decoloration effects. The recovery rate of polysaccharide was highest also. The values of that of D101 were 77.14, 78.7 and 79.56%, respectively separately. Results obtained in this study indicated that macroporous adsorption resin D101

would be a cost-effective, easily regenerated, environmentally friendly and easily upscaled method to purify the polysaccharide extracted by alkaline solution from perilla seed meal.

### ACKNOWLEDGMENT

This study is supported by the Science and Technology Research Project of Chongqing Municipal Education Commission of China (Grant No. KJ120734).

## REFERENCES

- Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72: 248-254.
- Cheison, S.C., Z. Wang and S.Y. Xu, 2007. Use of adsorption resin for simultaneous desalting and debittering of whey protein hydrolysates. *Int. J. Food Sci. Tech.*, 42: 1228-1239.
- Li, Q., J.D. Lei, R.Y. Zhang, J. Li, J.M. Xing, F. Gao, F.L. Gong, X.F. Yan, D. Wang, Z.G. Su and G.H. Ma, 2013. Efficient decolorization and deproteinization using uniform polymer microspheres in the succinic acid biorefinery from bio-waste cotton (*Gossypium hirsutum* L.) stalks. *Bioresource Technol.*, 135: 604-609.
- Li, Q.H., C.L. Fu, Y.K. Rui, G.H. Hu and T.Y. Cai, 2005. Effects of protein-bound polysaccharide isolated from pumpkin on insulin in diabetic rats. *Plant Food. Hum. Nutr.*, 60: 13-16.
- Liu, J., J.G. Luo, Y. Sun, H. Ye, Z.X. Lu and X.X. Zeng, 2010a. A simple method for the simultaneous decoloration and deproteinization of crude levan extract from *Paenibacillus polymyxa* EJS-3 by macroporous resin. *Bioresource Technol.*, 101: 6077-6083.
- Liu, Y.F., J.X. Liu, X.F. Chen, Y.W. Liu and D.L. Di, 2010b. Preparative separation and purification of lycopene from tomato skins extracts by adsorption resins. *Food Chem.*, 123: 1027-1034.
- Longvah, T. and Y.G. Deosthale, 1998. Effect of dehulling, cooking and roasting on the protein quality of *Perilla frutescens* seed. *Food Chem.*, 63: 519-523.
- Ma, C.Y., G.J. Tao, J. Tang, Z.X. Lou, H.X. Wang, X.H. Gu, L.M. Hu and M.L. Yin, 2009. Preparative separation and purification of rosavin in *Rhodiola rosea* by adsorption resins. *Sep. Purif. Technol.*, 69: 22-28.
- Masuko, T., A. Minami, N. Iwasaki, T. Majima, S.I. Nishimura and Y.C. Lee, 2005. Carbohydrate analysis by a phenol-sulfuric acid method in microplate format. *Anal. Biochem.*, 339: 69-72.
- Shin, H.S. and S.W. Kim, 1994. Lipid composition of perilla seed. *J. Am. Oil Chem. Soc.*, 71: 619-622.
- Tang, Z.G., R.Q. Zhou and Z.T. Duan, 2001. Adsorption and desorption behaviour of taurine on macroporous adsorption resins. *J. Chem. Technol. Biot.*, 76: 752-756.
- Tong, B. and D.C. Liu, 2008. Extraction of tannin and phytic acid from perilla seed meal. *China Oil. Fat.*, 33: 47-50.
- Wang, F., J.R. Li and Y.M. Jiang, 2010. Polysaccharides from mulberry leaf in relation to their antioxidant activity and antibacterial ability. *J. Food Process. Eng.*, 33: 39-50.
- West, T.P. and B. Reed-Hamer, 1993. Polysaccharide production by a reduced pigmentation mutant of the fungus *Aureobasidium pullulans*. *FEMS Microbiol. Lett.*, 113: 345-349.
- Yamamoto, H. and T. Ogawa, 2002. Antimicrobial activity of perilla seed polyphenols against oral pathogenic bacteria. *Biosci. Biotech. Bioch.*, 66: 921-924.
- Zhu, J.F. and Q. Fu, 2012. Optimization of ultrasound-assisted extraction process of perilla seed meal proteins. *Food Sci. Biotechnol.*, 21: 1701-1706.
- Zhu, J.F., S.L. Yang, G. Chen and C.H. Tang, 2011. Optimization of extracting technology of polysaccharide for perilla meal by alkali. *Food Res. Dev.*, 32: 69-71.