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Recovery of Astaxanthin from Discharged Wastewater During the Production of Chitin

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Abstract In this paper, studies were carried out to extract astaxanthin from discharged wastewater during the production of chitin and to reveal the scavenging effect of the obtained pigment on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical. Different ratios of dichloromethane/methanol (V/V) were used to extract astaxanthin. When the ratio of dichloromethane/methanol was 2:8 and the ratio between the mixed organic solvent (dichloromethane/methanol, 2:8, V/V) and wastewater was 1:1, the highest yield of pigment was obtained (8.4 mg/ 50 mL). The concentration of free astaxanthin in the obtained pigment analyzed by HPLC was 30.02%. The obtained pigment possessed strong scavenging ability on DPPH radical and IC_{50} was 0.84mg/ml.

Key words dichloromethane/methanol; astaxanthin; extract; pigment; wastewater

1 Introduction

Astaxanthin (3, 3'-dihydroxy- β , β '-carotene-4, 4'-dione) is a carotenoid pigment found in certain marine animals and plants such as fish, shrimp and algae (Boussiba *et al*., 1992). It is widely used due to its high antioxidative effects. Many studies suggest that it has about 10-fold higher antioxidative activity than β-carotene and up to 500 times higher activity than vitamin E (Dimascio *et al*., 1990; Shimidzu *et al*., 1996). The most important commercial application of astaxanthin is in the aquaculture industry. It is used in the formation of feed to provide typical muscle color, improve reproduction embryo development and protect cells against oxidative damage(Putnam *et al*., 1991; Parajo *et al*., 1996). For human health, astaxanthin might help to prevent and fight several human diseases. It can be effective in preventing UV-light photooxidation of lipids (O'Connor and O'Brien, 1998), age-related macular degeneration and cataracts(Papas, 1999), carcinogenesis of skin cells (Fuchs, 1998). Astaxanthin could also be beneficial to heart health by modifying blood levels of LDL and HDL cholesterol (Murillo, 1992; Guerin *et al*., 2003).

The process of extracting chitin from shrimp waste imposes serious environmental problems, especially disposal problems of wastewater containing astaxanthin and protein. The recovery of these valuable components from the wastewater would not only improve the economy for shrimp processors, but also minimize the pollution (Sochindra and Mahendrakar, 2005). Presently, there are very few studies reporting the recovery of astaxanthin from discharged wastewater during the production of chitin. In this paper, we present preliminary results aiming at the recovery of astaxanthin by the use of mixed organic solvent and the scavenging effect of the obtained pigment against 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical.

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2 Materials and Methods

2.1 Determination of the Optimal Ratio of Dichloromethane/Methanol

Five replicates of discharged wastewater (50mL) obtained during the production of chitin from Qingdao Baicheng Marine Living Resource Ltd. were prepared. The mixed solvents of dichloromethane/methanol (50mL, V/V) with the ratios of 2:8, 4:6, 5:5, 6:4 and 8:2 were mixed with the prepared five replicates wastewater, respectively. Then, the mixtures were placed for 30 minutes. The pigmented organic phase was separated from the aqueous phase using a separating funnel. The organic phase was concentrated via rotary evaporator. The organic solvent was recovered for reuse. A saponification procedure was applied to the concentrated organic phase (Stepnowski *et al*., 2004). The saponified pigment was

dried under N_2 conditions and the obtained pigment was weighed.

2.2 Determination of the Optimal Ratio Between Mixed Organic Solvent and Wastewater

Different doses of the mixed organic solvent of dichloromethane/methanol (12.5 mL, 25 mL, 50 mL, 100 mL and 200 mL) were mixed with the above five replicates of wastewater. The ratios of the mixed organic solvents to wastewater were 1:4, 1:2, 1:1, 2:1 and 4:1, respectively. Then, the mixtures were placed for 30 min. The pigmented phase was separated from the aqueous phase. The concentration and saponification of the pigmented organic phase was the same as in 2.1.

2.3 Analysis of the Obtained Pigment

The obtained pigment was analyzed by HPLC according to Oliver and Palou (2000). The HPLC system used consists of a Waters 515 pump (Waters, Milford, MA, USA), a sample injector (Rheodyne, Cotati, CA, USA) with 20 μL loop, and a Waters 996 photodiode array detector. Evaluation and quantification were made on a Millenium Chromatography data system (Waters). The C_{18} column (300*3.9mm, I. P., 5 μm, Beckman, Fullerton, CA, USA) was used. The mobile phase was methanol-water (9:1, V/V). The flow rate was 1.5 mL min^{-1} and the effluent was monitored at 475 nm. The area under the peak of free astaxanthin was calculated. Standard astaxanthin (Sigma, USA) was used.

2.4 Scavenging of DPPH Radical

The effects of the obtained pigment on DPPH radical were studied employing the modified method described earlier by Yamaguchi *et al*. (1998). Briefly, 1.5ml of DPPH solution (0.1 mmolL^{-1}, in methanol) was incubated with varying concentration of pigment (0.84, 1.1, 1.4, 2.1, 4.2 and 8.4 mg mL^{-1}, respectively, in methanol). The reaction mixture was well shaken and incubated for 30 min at room temperature and the absorbance of the resulting solution was read at 517 nm against a blank. The scavenging activity of DPPH radical was measured as a decrease in the absorbance of DPPH and was calculated using the following equation:

Scavenging effect (%) = (1-A samples $_{(517nm)}$/A control $_{(517nm)}$)×100.

2.5 Statistical Analysis

All determinations were carried out in triplicate. All data are expressed as means ± SD. Data were analyzed by an analysis of variance ($P<0.05$) with the means separated by Duncan's multiple range tests. The results were processed using Excel and statistical software (2003).

3 Results and Discussion

In Fig.1, when the ratio of dichloromethane/methanol (V/V) was less than 1, less and less pigment was obtained with the increase of the ratio. When the ratio of dichloromenthane/methanol exceeded 1, the obtained pigment increased slightly with the increase of the ratio. However, when the ratio of dichoromethane/methanol was 2:8, the yield of the obtained pigment was higher than the others. Hence, the ratio of 2:8 for dichoromethane/methanol was the optimal ratio.

In Fig.2, with the increase of the ratio between the mixed organic solvent and wastewater (V/V), more pigment was obtained. Nevertheless, when the ratio exceeded 1, the obtained pigment increased slowly with the increase of the ratio. Thus, the ratio of 1:1 between the mixed organic solvent and wastewater was the optimal ratio.

In Fig.3, the retention time of free astaxanthin in standard astaxanthin was 6.339. In Fig.4, the retention time of free astaxanthin in the obtained astaxanthin was 6.357. The areas under the peaks of free astaxanthin in stan-

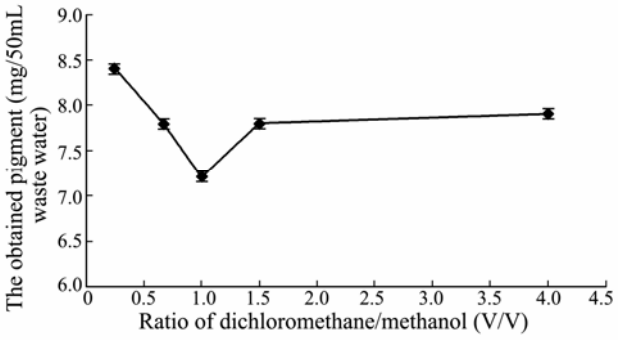

Fig.1 The effect of ratios of dichloromethane/methanol (V/V) on the obtained pigment.

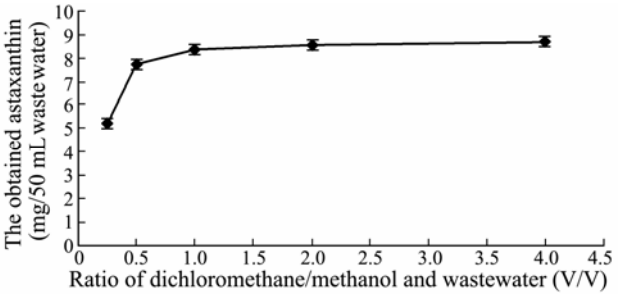

Fig.2 The effect of ratios of dichloromethane/methanol and wastewater (V/V) on the obtained astaxanthin.

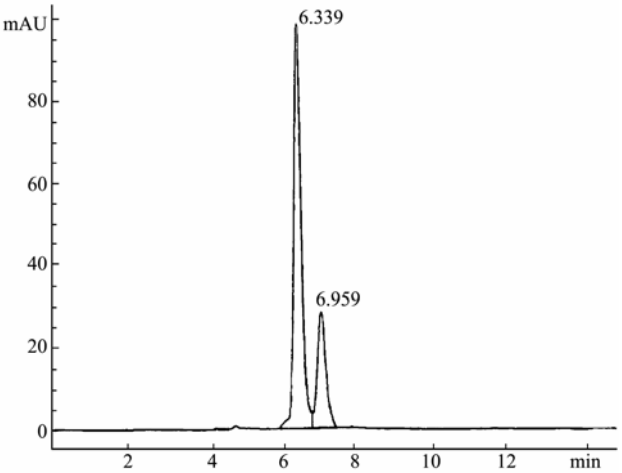

Fig.3 Chromatogram of standard astaxanthin (Sigma).

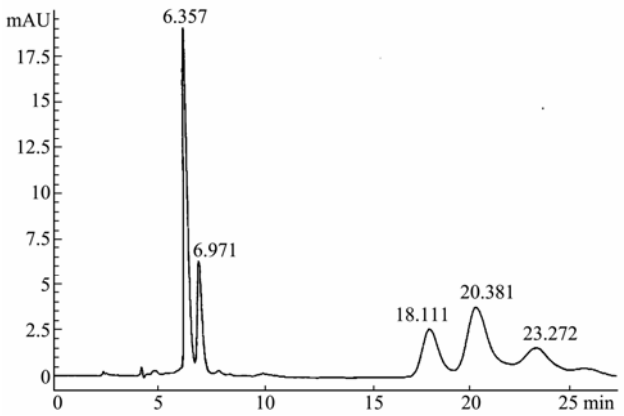

Fig.4 Chromatogram of the obtained pigment.

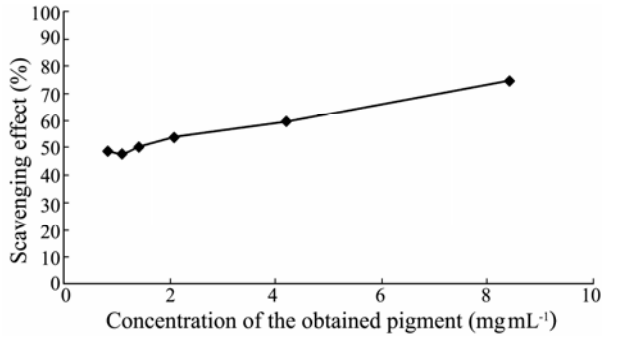

Fig.5 the effect of the obtained pigment on DPPH radial.

dard astaxanthin and in the obtained astaxanthin were calculated. According to the area and the content of free astaxanthin in standard astaxanthin, we concluded that the content of free astaxanthin in the obtained astaxanthin was 30.02%.

The scavenging potential of the obtained pigment at varied concentration was measured and the results were depicted in Fig.5. From Fig.5, the scavenging effect of the obtained pigment increased with the increase of the concentration. Obviously, the pigment possessed strong scavenging ability on DPPH radical and ID_{50} was 0.84 mg mL^{-1}. In the DPPH test, the obtained pigment was able to reduce the stable DPPH radical to the yellow-colored diphenyl-picrylhydrazine. DPPH is one of the compounds that possess a proton free radical with a characteristic absorption, which decreases significantly on exposure to proton radical scavengers. In the present study, the obtained pigment including 30.02% of free astaxanthin showed excellent scavenging activity of DPPH radical, which may be attributed to its strong donating ability.

In recent years, there has been increasing interest in finding natural antioxidants, since they can protect the human body from free radicals and retard the progress of many chronic diseases (Kinsella *et al*., 1993). The ability of astaxanthin to quench oxygen radicals has been studied (Cantrell *et al*., 2003). However, the scavenging effect of astaxanthin on DPPH of radicals was sparsely studied. Although astaxanthin has strong scavenging effect on free radicals and other essential biological functions, it can not be synthesized from animals and so far has not been widely used due to a limited number of suppliers and the high cost (Waldenstedt *et al*., 2003). The Hoffman-La roche Inc. has synthesized all-trans astaxanthin (Fang and Chiou, 1996). However, the biological function of synthesized astaxanthin is poorer than that of natural astaxanthin (Wei and Yan, 2001). Therefore, it is important to find new sources and novel manufacturing technology for obtaining astaxanthin. In this paper, we utilized the mixture of dichloromethane and methanol to extract astaxanthin. This process extractes astaxanthin quickly and effectively. Furthermore, it avoids the decreasing yield of astaxanthin due to high temperature and has advantages in both environmental and economical considerations. However, the purification and application of the obtained pigment need to be further researched.

Conclusions based on the present study are summarized in the 'Abstract' of the paper.

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