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Three-liquid-phase salting-out extraction of effective components from waste liquor of processing sea cucumber

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ABSTRACT

A three-liquid-phase (TLP) salting-out extraction system composed of *n*-hexane/ethanol/sodium carbonate/water was investigated to extract oils, saponins, proteins and polysaccharides simultaneously from waste liquor of processing sea cucumber. The effects of the ratio of ethanol to sodium carbonate, *n*-hexane concentration and extraction time were investigated. The results showed that 86.7% of oils were distributed in the top *n*-hexane phase, 82.9% of saponins in the middle ethanol phase, 93.2% of proteins and 92.9% of polysaccharides in the interface between the middle and the bottom salt phase when the system composed of 28% (w/w) *n*-hexane/11.52% (w/w) ethanol/8.64% (w/w) sodium carbonate was used at 37 °C for 0.5 h. When the system was progressively enlarged from 30 g to 25 kg, the yield of polysaccharides, proteins, oils and saponins was decreased only by 1.0%, 2.8%, 1.0% and 2.6%, respectively. The recycle of salt and solvents in three-liquid-phase system was also studied, and the results showed that the recovery of *n*-hexane, ethanol and salt were 81.4%, 80.8% and 72.0%, respectively. Recycling materials for the extraction, the yield for proteins and oils decreased by 2.0% and 5.4%, respectively, comparing with the pure system.

1. Introduction

Sea cucumber is a kind of precious seafood, known as "sea ginseng". It contains more than 50 kinds of nutrients including amino acids, essential or polyunsaturated fatty acids, vitamins and trace elements, etc., and active substances, such as collagen proteins, polysaccharides, saponins and brain glycosides (Jiang et al., 2004). These substances have many roles of antitumor (Jin et al., 2009; Sugawara et al., 2006), anticoagulant (Li and Lian, 1988), lowering blood-fat (Liu et al., 2012), antifungal activity (Chludil et al., 2002; Kumar et al., 2007), inhibition of angiogenesis (Zhao et al., 2011), immunomodulatory (Wang et al., 2010), inducing neurite outgrowth (Kaneko et al., 2007). Therefore, sea cucumber has a wide range of applications in fields of medicine, health care, food, etc. Due to easy autolysis of sea cucumber out of the sea water and the need for preservation, more than 90% of the fresh sea cucumbers are usually processed into instant, semi- or dried products. As the first step of the process, heating and cooking are normally used to deactivate the autolyzed enzymes in sea cucumbers. After then a large amount of the processing liquor is produced as waste byproduct to discharge into sea. The chemical compositions in the waste liquor are almost the same as sea cucumber itself, such as polysaccharides, proteins, oils, saponins and so on. If the active components were separated effectively from the waste liquor, pollution of the

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environment would be reduced and considerable economic benefits could be obtained. The methods for extracting active ingredients from waste liquor of processing sea cucumber include precipitation of protein at their isoelectric point, alcohol precipitation, desalination and distillation (Zhao et al., 2010; Yuan et al., 2009; Jiang, 2012; Cong et al., 2006). Usually only one or two kinds of active substances were extracted or separated from the waste liquor. However, other effective components were not considered synchronously. As these mentioned methods indicated, multi-step separation technologies were applied to separate multi-active substances from the processing sea cumber, which led to the loss of active substances and high cost of recovery.

Multiphase salting-out extraction can achieve the recovery of the multi-active substances synchronously with simple and effective step. The multiphase salting-out extraction system is composed of hydrophobic/hydrophilic organic solvent, salt and water. The ingredients in a complex sample will distribute into the different phases of multiphase salting-out extraction system according to their quality in polarity and solubility, e.g. water-soluble, alcohol-soluble and fat-soluble. Multiphase salting-out extraction systems have many advantages, such as short time to form phase due to low viscosity of system, easy recovery of solvent by evaporation, low cost, simple scale-up and high product yield (Xu and Wang, 2009). The system is also known as aqueous two-phase system in this multiphase salting-out extraction system when the hydrophilic solvent is only used. If the solvents are composed of hydrophilic and hydrophobic solvents, the system is also called three-liquid-phase system. The aqueous two-phase system have been successfully used in separation of bio-based chemicals, e.g. 1,3-propanediol, 2,3-butanediol, biobutanol, lactic acid and succinic acid (Liu et al., 2009; Jiang et al., 2009; Sun et al., 2009, 2014; Li et al., 2009, 2010, 2011; Dai et al., 2011), natural products, e.g. α-galactosidase, oil and alginates and recombinant human albumin (Dhananjay and Mulimani, 2009; Sharma et al., 2002; Sharma and Gupta, 2002; Dong et al., 2012). The three-liquid-phase salting-out extraction has been applied in the isolation of complex natural products e.g. diosgenin and steroidal saponins (Liu et al., 2010; Wei et al., 2012). In our previous report, aqueous two-phase salting-out extraction system can successfully achieve extraction of polysaccharides and proteins from waste liquor (Chen et al., 2013). The results indicated that *t*-butanol/ammonium sulphate and ethanol/sodium carbonate systems performed better in extracting protein and polysaccharide directly from the waste liquor. The recovery yield of protein for two different aqueous two-phase systems can reach 99.6% and 96.9%, and the recovery yield of polysaccharide were 96.3% and 90.6%, respectively. In previous study, both saponins and oils mainly existed in the solvent phase when a single hydrophobic or hydrophilic solvent used in salting-out extraction system. Other separation technology was still need to further separate saponins and oils from the solvent phase. Therefore, in this study a three-liquid-phase salting-out extraction system containing ethanol, sodium carbonate and hexane was applied to extract proteins, polysaccharides, saponins, oils simultaneously from waste liquor. The effects of different *n*-hexane concentration and extraction time on the partition of the proteins, polysaccharides, saponins, oils were investigated to determine the optimal extraction conditions for three-liquid-phase salting-out extraction system. In addition, amplification of this three-liquid-phase system and the recycle of solvents and salt were also investigated.

2. Materials and methods

2.1. Materials and reagents

Waste liquor of processing sea cucumber was provided by a marine biotechnology company in Dalian, Liaoning Province, China. Its dry weight ratio was 5.03% and the amounts of proteins, polysaccharides, saponins and oils in the freeze-dried powder were 15.51, 11.07, 1.69, and 3.70%, respectively. Astrageloside III (HPLC purifity ≥ 98%) was purchased from Sichuan Camila Vickerage Biological Technology Co., Ltd., China. Vanillin purchased from Tianjin Kermel Chemical Reagent Co., Ltd. was of analytical grade. 3,5-Dinitrosalicylic acid purchased from Sinopharm Chemical Reagent Co., Ltd. was of chemically pure. All other chemicals were of analytical grade.

2.2. Analysis methods

Kjeldahl method (GB5009.5-85) was used to measure the content of total proteins. The Coomassie Brilliant Blue method was used to measure the concentration of soluble proteins, using BSA as a standard protein (Bradford, 1976). The total polysaccharides equal to total reducing sugars minus reducing sugars existing in the original waste liquor which were measured at 550 nm by a spectrophotometer, using glucose as a standard sugar (Miller, 1959).

Crude polysaccharides were measured by the method of alkali dissolution and alcohol precipitation as follows. 0.500 g of freeze-dried powder were added into 80 mL 5% NaOH solution, dissolved 2 h under ultrasound treatment, then glacial acetic acid was added to adjust the pH to 7.0 for precipitation. The solution was spun down at 8000 rpm for 15 min, the supernatant was added to twice the volume of 95% ethanol and spun down at 8000 rpm for 15 min. The precipitate was taken to freezing-dry.

Saponins were measured by vanillin-perchloric acid method at 578 nm, using Astrageloside III as a standard saponin (Dong et al., 2008). The standard curve equation was $Y = 1.39x + 0.0032$ and the linear correlation coefficient was 0.9992.

Soxhlet extraction (GB/T 5009.6-2003) was used to determine the oils content in the dry powder. Gravimetric method was used to determine the oils content in the organic phase, weighing after evaporation of the solution.

The alcohol meter (Li Dwellings Standard Glass Instrument Factory, Hejian City, China) was used to measure the concentration of the reclaimed ethanol.

The salt concentration was determined on basis of the following relationship: salt concentration vs. its conductivity was a linear relationship in a certain range of salt concentrations. Therefore, the determination of the salt concentration in three-liquid-phase system was finished with DDS-11A digital conductivity meter (Shanghai Tianda Instrument Co., Ltd., China).

2.3. Experiments

2.3.1. Three liquid-phase salting-out extraction

The three-liquid-phase salting-out extraction system was composed of *n*-hexane, ethanol, sodium carbonate and waste liquor with total mass of 30 g. A certain amount of waste liquor was added to 50 mL graduated centrifuge tube with stopper,