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Research Article

Study on the Isolation and Purification Process of Fucoidan from Sea Cucumber Blanching Liquid Waste

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Abstract:

Shandong Province regards marine biomedicine as the core direction for the high-quality development of the marine economy. The blanching liquid produced during sea cucumber processing is often directly discharged in large quantities, which on one hand causes resource waste and environmental pollution, and on the other hand, presents a challenge for the low-cost, large-scale preparation of sea cucumber fucoidan with high anti-Helicobacter pylori activity. This paper elaborates on the theoretical basis for the isolation and purification of sea cucumber fucoidan, systematically optimizes key processes such as multi-stage membrane separation, composite enzymatic hydrolysis-ethanol precipitation, and CPC (Cetylpyridinium chloride) precipitation-chromatography. Furthermore, it explores strategies for molecular weight homogenization and activity retention, providing technical support for the high-value utilization of blanching liquid and laying the foundation of high-quality raw materials for the development of anti-Helicobacter pylori functional products.

Keywords:

Sea cucumber blanching liquid; Fucoidan; Isolation and purification; CPC precipitation; Anti-Helicobacter pylori

Introduction

The blanching liquid from sea cucumber processing is rich in active substances such as fucoidan but is often discarded, leading to both resource waste and environmental pollution. This study focuses on the theory and processes of isolating and purifying sea cucumber fucoidan, constructing an efficient and green preparation technology. This provides support for the high-value utilization of marine biological resources and the development of anti-Helicobacter pylori products.

1. Theoretical Basis for the Isolation and Purification of Sea Cucumber Fucoidan

1.1 Chemical Characteristics and Biological Activities of Fucoidan

Fucoidan is a type of polysaccharide that is mainly composed of L-fucosyl groups and sulfate groups. It belongs to water-soluble sulfate polysaccharides and is widely present in brown algae and echinoderms. Compared with the fucoidan derived from seaweed, the fucoidan from sea cucumbers has unique chemical structural characteristics. Its main chain is generally composed of α-1,3-linked fucosyl residues. Some of the residues have sulfate groups at C-2 or C-4 positions, while it also contains small amounts of monosaccharides such as glucuronic acid, galactose, and xylose. This special structure endows fucoidan from sea cucumbers with excellent biological activities, including antioxidant, anti-inflammatory, anti-tumor, immune regulation, and the anti-Helicobacter pylori activity that is the focus of this project.

Studies have shown that the biological activity of sea cucumber fucoidan is closely related to its molecular weight, sulfate group content and position, and monosaccharide composition. Among these, the sulfate groups and fucose are key active groups for its anti-Helicobacter pylori effect, as they can specifically bind to the mucins on the surface of Helicobacter pylori, thereby preventing its colonization on the gastric mucosa. In addition, as an animal-derived saccharide, sea cucumber fucoidan possesses better molecular structural flexibility and biocompatibility. Its affinity for human gastric mucosal cells is significantly higher than that of seaweed-derived fucoidan, which is a major reason for its stronger anti-H. pylori activity.

1.2 Composition and Resource Value of Sea Cucumber Blanching Liquid Waste

The blanching liquid is wastewater produced during sea cucumber processing, generated after fresh sea cucumbers are blanched in hot water at 80-100°C for 3-5 minutes. Approximately 5-8 tons of wastewater are produced per ton of fresh sea cucumber. Its dry matter content is 1%-3%, mainly consisting of polysaccharides (30%-40% of dry weight, with fucoidan accounting for 20%-30%), proteins and peptides (25%-35%), free amino acids, minerals, and small amounts of lipids and nucleotides. For a long time, this wastewater has been directly discharged, which not only wastes resources but also increases sewage treatment costs. Comprehensive utilization and recovery of its high-value active ingredients can transform waste into

treasure, improve corporate efficiency, and reduce pollution, aligning with the concept of green sustainable development.

1.3 Principles and Methodological Basis of Isolation and Purification Technology

The isolation and purification of polysaccharides is a complex process comprising four stages: pretreatment, crude extraction, preliminary purification, and fine purification. Pretreatment involves removing insoluble impurities through centrifugation and filtration. Crude extraction utilizes methods such as water extraction or enzymatic extraction to release polysaccharides. Preliminary purification involves deproteinization, decolorization, and desalinization to remove most non-polysaccharide impurities. Fine purification uses techniques like chromatography and electrophoresis to obtain high-purity, homogeneous polysaccharide components.

Current major technologies for the isolation and purification of sea cucumber fucoidan include: membrane separation (using membranes of different pore sizes to selectively permeate substances of different molecular weights for desalinization, concentration, and initial fractionation); enzymatic hydrolysis (specifically degrading protein impurities via proteases to improve purity); ethanol precipitation (utilizing the decreased solubility of polysaccharides in high-concentration ethanol for precipitation and separation); Cetylpyridinium chloride (CPC) precipitation (using cationic surfactants to form insoluble complexes with negatively charged sulfated polysaccharides to separate them from neutral polysaccharides); and ion-exchange chromatography and gel filtration chromatography (separating based on differences in polysaccharide charge and molecular weight, respectively). Each of these technologies has its advantages and disadvantages, necessitating rational combination and optimization based on raw material characteristics and product requirements.

2. Optimized Process for Efficient Isolation and Purification of Fucoidan

2.1 Synergistic Process of Multi-stage Membrane Separation, Decolorization, and Deodorization

Multi-stage membrane separation technology is the core technology for achieving continuous and large-scale treatment of sea cucumber blanching liquid. This study constructed a three-stage membrane separation process consisting of "microfiltration-ultrafiltration-nanofiltration" to achieve integrated treatment for clarification, desalinization, concentration, and preliminary fractionation: first, a 0.22μm microfiltration membrane is used to remove suspended particles and macromolecular impurities to obtain a clarified filtrate; next, an ultrafiltration membrane with a molecular weight cut-off of 10kDa is used for concentration and the removal of most small-molecule salts, free amino acids, and monosaccharides; finally, a nanofiltration membrane further removes residual salts and small-molecule impurities to obtain a concentrated sea cucumber glycopeptide solution. To address the dark color and heavy fishy odor of the blanching liquid, this study

developed a synergistic decolorization and deodorization process combining activated carbon adsorption with macroporous resin. Through single-factor experiments and response surface methodology, key parameters such as activated carbon dosage, adsorption temperature, adsorption time, and macroporous resin type and flow rate were optimized. The results showed that with an activated carbon dosage of 1.5%, adsorption temperature of 50°C, and adsorption time of 30min, followed by AB-8 macroporous resin column chromatography, the decolorization rate reached over 85%, the deodorization effect was good, and the fucoidan retention rate remained above 90%. This process is simple to operate, relatively low-cost, and suitable for large-scale industrial application.

2.2 Combined Technology of Composite Enzymatic Hydrolysis and Ethanol Precipitation to Remove Non-polysaccharide Impurities

Protein is the primary non-polysaccharide impurity in sea cucumber glycopeptide powder, and its content directly affects the subsequent purification efficiency and product quality of fucoidan. A single enzymatic hydrolysis method is usually insufficient to completely remove proteins and may cause polysaccharide degradation. This study employed a composite enzymatic hydrolysis method using papain and trypsin, leveraging the synergistic effect of the two enzymes to efficiently degrade protein impurities.

Orthogonal experiments were conducted to optimize the composite enzymatic hydrolysis conditions: enzyme dosage was 2% of the substrate mass, the ratio of papain to trypsin was 2:1, hydrolysis temperature was 55°C, pH was 7.0, and hydrolysis time was 4h. Under these conditions, the protein removal rate reached 82%, while the fucoidan loss rate was below 10%. After removing insoluble proteins from the hydrolysate via centrifugation, fractional ethanol precipitation was used for preliminary separation. At a final ethanol concentration of 30%, sea cucumber chondroitin sulfate primarily precipitated; when the concentration reached 60%, most of the sea cucumber fucoidan was precipitated. By controlling the ethanol concentration and precipitation time, the preliminary separation of sea cucumber fucoidan and chondroitin sulfate was achieved, laying the foundation for subsequent fine purification.

2.3 Construction of CPC Precipitation-Chromatography Purification System

CPC precipitation is an effective method for isolating sulfated polysaccharides. Its principle is that CPC, as a cationic surfactant, forms an insoluble complex with negatively charged sulfated polysaccharides, while neutral polysaccharides and other impurities remain in solution. This study optimized the CPC precipitation process parameters: the CPC solution concentration was 10%, the added volume was 15% of the polysaccharide solution volume, the precipitation temperature was 25°C, and the time was 2h. Under these conditions, the fucoidan precipitation rate exceeded 90%. After the complex was dissociated with sodium

chloride and precipitated with ethanol, a crude fucoidan product with a purity of approximately 50% was obtained.

To enhance the purity of the fucoidan, this study constructed a two-step purification system of "ion-exchange chromatography - gel filtration chromatography": first, a Q-Sepharose Fast Flow anion-exchange column was used with a linear gradient elution of 0-2mol/L NaCl. The fucoidan main peak fractions were collected based on elution curves detected by the phenol-sulfuric acid method. After dialysis and freeze-drying, a Sephacryl S-400 gel filtration column was used with 0.2mol/L NH$_4$HCO$_3$ as the eluent at a flow rate of 0.5mL/min to collect symmetrical peaks. The final obtained sea cucumber fucoidan purity exceeded 70%, meeting the project's technical indicators.

2.4 Process Optimization for Molecular Weight Homogenization and Activity Retention

Sea cucumber fucoidan has a wide molecular weight distribution, and different fractions exhibit significant differences in biological activity. To obtain products with high activity and uniform molecular weight, this study utilized controllable enzymatic hydrolysis technology to regulate the molecular weight of the purified fucoidan. Components of different molecular weight ranges were prepared by screening suitable enzymes and optimizing parameters such as hydrolysis temperature, pH, enzyme dosage, and time. Simultaneously, this study focused on the retention of fucoidan activity during the isolation and purification process. By comparing the effects of different processes on sulfate group content and anti-Helicobacter pylori activity, it was found that harsh conditions such as high temperature, strong acids, or strong bases could cause the stripping of sulfate groups and the breakage of polysaccharide chains, significantly reducing biological activity. Therefore, the process design should employ mild treatments such as low-temperature concentration and neutral pH conditions for enzymatic hydrolysis and chromatography to maximize the preservation of structural integrity and biological activity. In vitro experiments showed that the sea cucumber fucoidan prepared by this optimized process has a significant inhibitory effect on the growth and adhesion of Helicobacter pylori, with activity comparable to that extracted directly from the sea cucumber body wall.。

Conclusion

Addressing the demand for the resource utilization of sea cucumber blanching liquid waste, and based on the theories of sea cucumber fucoidan isolation and purification, this study optimized and constructed a complete set of processes including multi-stage membrane separation, composite enzymatic hydrolysis, and combined chromatography. This achieved the efficient preparation of high-purity fucoidan while effectively retaining its anti-Helicobacter pylori activity. This process can solve the problems of pollution and resource waste caused by blanching liquid, reduce the cost of fucoidan preparation, provide technical support for the high-value

utilization of sea cucumber processing by-products, lay a foundation for the research and development of anti-Helicobacter pylori functional products, and contribute to the high-quality development of the marine biomedical industry.

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