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Recovery of valuable marine compounds from cuttlefish by-product hydrolysates: Combination of enzyme bioreactor and membrane technologies

Fractionation of cuttlefish protein hydrolysates by ultrafiltration: Impact on peptidic populations

Récupération de composés marins à haute valeur ajoutée à partir des hydrolysats de co-produits de la seiche : couplage bioréacteur enzymatique et procédés membranaires

Emna Soufi-Kechaou [a, b, *], Maryse Derouiniot-Chaplin [a], Raja Ben Amar [b], Pascal Jaouen [a], Jean-Pascal Berge [c]

[a] *Nantes University, GEPEA-UMR CNRS 6144, B.P. 406, Saint-Nazaire, France*
[b] *Laboratory of the Sciences of Material and Environment, Faculty of Sciences of Sfax, Tunisia*
[c] *Laboratory of the Sciences and Technology of Marine Biomass, Ifremer, Nantes, France*

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ABSTRACT

Recognition of limited resources and increasing environmental pollution has emphasized the need for better utilization of fisheries by-products (e.g., heads, frames, and viscera). The present investigation explores the technical feasibility of "clean technologies", that is, enzymatic hydrolysis and ultrafiltration to fractionate and concentrate *Sepia* by-products. The selected ultrafiltration membranes with intermediate molecular weight cut off allowed fractionating hydrolysates in several fractions of different molecular weights. The combined study of chromatographic data and amount of peptides quantified in permeates and retentates allowed us to study the progression of the fractionation in correlation with the increase or decrease in interesting molecules.

RÉSUMÉ

La raréfaction des ressources protéiques ainsi que l'attention particulière accordée à l'environnement ont amplifié le besoin d'une meilleure gestion des co-produits issus des pêcheries et des industries de transformation. Ce travail étudie la faisabilité technique de l'application de l'hydrolyse enzymatique en bioréacteur, et l'ultrafiltration pour le fractionnement des viscères de la seiche *Sepia officinalis*. Les membranes sélectionnées avec un seuil de coupure intermédiaire ont permis d'obtenir des fractions intéressantes de différents poids moléculaires.

* Corresponding author.
E-mail address: esoufi@gmail.com (E. Soufi-Kechaou).

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La combinaison des données chromatographiques avec la concentration en peptides quantifiés dans les perméats et les rétentats nous ont permis d'étudier la progression du fractionnement en rapport avec l'existence de molécules d'intérêt.

1. Introduction

Global production from capture fisheries and aquaculture supplied about 130 million tonnes of fish per year [1]. Seventy six percent (100 million tonnes) of this catch is intended for human consumption. However, depending on fish species and processing (canning, freezing, and so forth), only 50–70% of this catch is really used in human diet. The remainder, such as fishery by-products (e.g., heads, frames, skin, and viscera), are especially converted into animal feed and oil. However, this underutilized material has a nutritional value almost as good as whole fish [2]. Although there are many approaches to further utilize these resources, interest has been expressed in isolating or processing value-added components [3].

Cephalopods are an important economic resource for global fisheries. The cuttlefish (*Sepia officinalis)* is among the most exploited marine species in the Mediterranean and Atlantic waters. In the Mediterranean, the main resource of cuttlefish is located in the Gulf of Gabes (southeast of Tunisia), and the landings occur essentially in the fishing port of Sfax. The most important fisheries of cuttlefish in the northeast Atlantic waters are found in the "La Manche" channel, where *S. officinalis* comprised 66% of the total cephalopods landed in Basse-Normandie, which is the foremost producing region in France. During transformation steps in seafood plants, large quantities of waste, including viscera, are generated and discarded. It is estimated that 40% of the total body weight ends up as a processing by-product that is not utilized, causing a serious disposal problem. Traditionally, marine viscera have been considered as waste and have been utilized only to a minor extent [4]. Nowadays, marine by-product hydrolysates are widely investigated for their biological activities, and different purification ways are used for that purpose [5,6].

In the recent years, cephalopoda have been studied only to a minor extent in terms of enzymatic hydrolysis. The cuttlefish *S. officinalis* viscera have been studied only in terms of autolysis [7]. However, fish enzymes have been widely studied in terms of structural modification, functional properties, biological activities, and lipid and phospholipid recoveries [8–12].

In recent years, a large number of biologically active peptides have been generated from fishery waste and by-products (e.g., heads, frames, and viscera). Consequently, interesting and very promising new applications for the fish and shrimp by-product hydrolysates have emerged. For example, active factors such as peptides inhibiting the angiotensin I-converting enzyme (thus exhibiting an antihypertensive effect), gastrointestinal peptides such as gastrin and cholecystokinins 9–10, cellular growth factors11, factors such as calcitonin and calcitonin gene-related peptide 12 were detected in hydrolysate fractions. The presence of antioxidant compounds in marine hydrolysates has also been reported [13].

Production of fish protein hydrolysate (FPH) by proteinase treatment is a mean to transform by-products into products with improved functional and biological properties. Indeed, appropriate hydrolysis parameters can produce hydrolysates with different biological activities (e.g., antihypertensive, immunostimulatory, antioxidant, and so forth).

Enzymatic protein hydrolysis is a promising process for underutilized marine products. FPHs from various sources have been studied extensively and described by several researchers [14]. Enzymatic hydrolysis allows the production of small peptides and even free amino acids [15].

The molecular weight (MW) of the hydrolyzed proteins is one of the most important factors in producing protein hydrolysates with bioactive peptides. Accordingly, to obtain peptide fractions with desired molecular size, we have used ultrafiltration (UF) and nanofiltration (NF) systems. These processes used porous membranes characterized by the molecular weight cutoff (MWCO), which indicates the smallest MW component retained at 90% [16]. UF and NF aim at concentrating and/or fractionating one or several components of a solution to permit selective passage of one or several of these components through the membrane.

In the present study, the potential of various membranes to fractionate an FPH using UF and NF membranes is described in a first step. In a second step, a process integrating appropriate membranes was applied to fractionate and concentrate.

2. Materials and methods

2.1. Materials

The spray-dried cuttlefish Protein hydrolysates (CPH) ($0.1 < MW < 10$ kDa) were obtained by Protamex, Alcalase, and Flavourzyme bioreactor hydrolysis at the laboratory.

2.1.1. Biological material

Cuttlefish (*S. officinalis*) were provided by the seafood processing company "Calembo" (Sfax, Tunisia). The cephalopods were then immediately stored in ice and transported to the laboratory where they were eviscerated. The collected viscera were homogenized for 1 min and then frozen at −80 °C until used. Endogenous enzymes were not inactivated. The cuttlefish viscera fraction included all of the organs usually found in the abdomen of mature specimens, that is, the digestive gland, esophagi, stomach, digestive ducts, pyloric caeca, pancreatic diverticula, gonads, and accessory nidamental glands; only the ink gland was removed.