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# Chemical and biological characteristics of protein hydrolysates from fermented shrimp by-products

C. Bueno-Solano [a], J. López-Cervantes [b], O.N. Campas-Baypoli [a], R. Lauterio-García [a], N.P. Adan-Bante [a], D.I. Sánchez-Machado [b,*]

[a] Doctorado en Ciencias en Biotecnología, Instituto Tecnológico de Sonora, 5 de Febrero 818 Sur, CP 85000, Cd. Obregón, Sonora, Mexico
[b] Departamento de Biotecnología y Ciencias Alimentarias, Instituto Tecnológico de Sonora, 5 de Febrero 818 Sur, CP 85000, Cd. Obregón, Sonora, Mexico

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ABSTRACT

Protein hydrolysates were prepared through lactic acid fermentation of the inedible portions of shrimp (cephalothorax and exoskeleton), the by-products of shrimp processing operations. The protein-rich liquid hydrolysate was further processed into a concentrated paste via vacuum evaporation at 80 °C or was also processed into a dry powder using a spray drying method at 180 °C/140 °C (inlet/outlet temp). The laboratory compared the composition of the three forms of shrimp protein hydrolysates. The protein and ash content of the hydrolysates ranged from 8.43 ± 0.22 to 46.73 ± 1.29 and 2.03 ± 0.52 to 8.25 ± 0.14 g/100 g of wet weight. All the samples were analyzed for fifteen amino acids; the powder form was analyzed for colour, microbial content, and for heavy metal occurrence. The shrimp by-products were successfully converted into micro-nutrient by-products rich in amino acids for potential recommendations in the supplementation of animal and human diets.



## 1. Introduction

Protein hydrolysates have a variety of applications in a variety of industries, including pharmaceuticals, human nutrition, animal nutrition, or cosmetics. Protein hydrolysates are also useful as a nitrogen source in the growth media for microorganisms (Duarte de Holanda & Netto, 2006; Quitain, Sato, Daimon, & Fujie, 2001). Recently, protein hydrolysates, extracted from marine by-products, have become popular in the food industry due to the high protein content (Córdova-Murueta, Navarrete-del-Toro, & García-Carreño, 2007). Protein hydrolysis refers to any process in which the protein is broken down by protein-digesting enzymes (Vidotti, Macedo-Viegas, & Carneiro, 2003). Hydrolysis decreases the peptide size, making hydrolysates the most available amino acid source for protein biosynthesis (Gildberg & Stenberg, 2001).

Shrimp by-products have been identified as an animal protein source of great potential; also, as an important source of chitin and asthaxanthin (Shahidi & Synowiecki, 1991). Only 65% of the shrimp is edible. The remainder is discarded as inedible waste (cephalothorax and exoskeleton). Over the years, techniques have been developed for the exploitation and recovery of these by-products in valuable biopolymers (Ferrer, Paez, Marmol, Ramones, Garcia & Forster, 1996).

Researchers have commonly utilized sun-drying as a recovery method to preserve the shrimp cephalothorax, but is often carried out under unhygienic conditions (Nwanna, Balogun, Aenifuja, & Enujiugha, 2004). Also, formic acid has been used for the protein recovery of these by-products (Nwanna, 2003). Some hydrolysis chemicals have reportedly been applied to the shrimp by-products, for example, 1 M HCl (Ferrer et al., 1996 and Kjartansson, Zivanovic, Kristbergsson, & Weiss, 2006), sodium sulphite (Mizani, Aminlari, & Khodabandeh, 2005) and NaOH and KOH for alkaline deproteinization (Duarte de Holanda & Netto, 2006). However, the use of strong acids or strong bases makes this process ecologically unacceptable. Accelerated hydrolysis, accomplished by the use of commercial enzymes such as papain, trypsin, pepsin (Chakrabarty, 2002), alcalase (Gildberg & Stenberg, 2001 and Synowiecki & Al-Khateeb, 2000), neutrase and protease (Ruttanapornvareesakul et al., 2006), has many advantages since accelerated hydrolysis allows for the control of the hydrolysis and thus minimizes undesirable reactions.

As a substitute to the chemical and enzymatic process, the lactic fermentation process for decomposition has been evaluated as a positive procedure (Armenta-López, Guerrero, & Huerta, 2002). Fermentation represents a cheap technique which will stabilize and retain the nutritional quality of the by-products (Fabgenro & Bello-Olusoji, 1997). Lactic fermentation has been used for red crab shell (Jung, Kuk, Kim, & Park, 2005), crayfish shell (Bautista et al., 2001), shrimp by-products and scampi by-products. With lactic fermentation, technicians can recover such components of the

* Corresponding author. Tel.: +52 644 4100900; fax: +52 644 4109001.
*E-mail address:* dsanchez@itson.mx (D.I. Sánchez-Machado).

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by-products as protein hydrolysate, chitin, minerals, and lipids (López-Cervantes, Sánchez-Machado, & Rosas-Rodríguez, 2006).

The purpose of this study was to examine the chemical and biological characteristics of protein hydrolysate from fermented shrimp by-products prepared under two processes, spray drying into a powder and the formation of a paste by concentrating the liquid hydrolysate with heat at 80 °C. Spray drying, the method used for converting a liquid into powder, has been employed to obtain hydrolysate from black tilapia (Abdul-Hamid, Bakar, & Bee, 2002); however, there are no reports from spray drying shrimp by-products. The principal substances, examined in the protein hydrolysates, were the amino acid composition, protein and ash content, colour, microbial flora and heavy metal content. This study is part of an investigation focused on a complete nutritional characterisation of the protein hydrolysate from shrimp by-products.

## 2. Materials and methods

### 2.1. Chemicals

HPLC-grade methanol and acetonitrile were obtained from EMD Chemicals (Darmstadt, Germany). Glacial acetic acid, boric acid, anhydrous ammonium monohydrogen phosphate, anhydrous dihydrogen phosphate, sodium hydroxide, sulphuric acid, ethylenediaminetetraacetic acid (EDTA) and HCl were all obtained from Products Monterrey (Monterrey, Nuevo León, México).The selenium reagent mixture was obtained from Merck (Darmstadt, Germany). The amino acid standard, hydroxylamine hydrochloride, 9-fluorenylmethyl chloroformate (FMOC–Cl) and 2(methylthio)-ethanol were purchased from sigma (St. Louis, MO, USA). All the reagents were analytical grade. All aqueous solutions were prepared with ultrapure water purified with a NANOpure Diamond UV system (Barnstead International, Dubuque, Iowa, USA). The ammonium monohydrogen phosphate, $(NH_4)_2HPO_4$, stock solution (2 M), used for preparation of HPLC eluents, was adjusted to pH 6.5 with ammonium dihydrogen phosphate, $NH_4H_2PO_4$.

Fifteen amino acids were detected in the sample: aspartic acid (Asp), glutamic acid (Glu), serine (Ser), glycine (Gly), histidine (His), arginine (Arg), threonine (Thr), alanine (Ala), proline (Pro), tyrosine (Tyr), valine (Val), methionine (Met), isoleucine (Ile), leucine (Leu) and phenylalanine (Phe). Amino acid standards were dissolved in 0.25 M borate buffer (pH 8.5). All samples were analyzed in duplicate.

These solutions were used for the derivatization of amino acids. FMOC–Cl was dissolved in acetonitrile at 4 mg/ml. A borate buffer was prepared from a 250 mM boric acid solution adjusted to pH 8.5 with 1 M sodium hydroxide solution prepared from sodium pellets. The alkaline cleavage reagent was prepared daily in 1000 μl batches by mixing 680 μl of 850 mM sodium hydroxide solution with 300 μl of 500 mM hydroxylamine hydrochloride solution and 20 μl of 2-(methylthio)ethanol. The quench reagent was acetonitrile-acetic acid (8:2, v/v).

### 2.2. Preparation of liquid protein hydrolysate

In the production of liquid protein hydrolysate, shrimp (*Penaeus* spp.) by-products samples (heads and cephalothoraxes) were collected from local shrimp processing factories in South Sonora, México. The by-products were packed in plastic bags and stored at −20 °C before the analysis procedure. Slightly thawed minced by-products were placed into 1000 ml glass flasks and mixed with 10% (w/w) cane sugar and 5% (v/w) commercial inoculum (optical density of cell = 1.7), stirred, and incubated in a water bath at 30 °C for 36 h. The silage was centrifuged (5 °C) at 1250 rpm for 15 min to obtain the chitin-rich fraction (sediment), the liquid protein hydrolysate, and the lipid fraction, Fig. 1. The liquid protein hydrolysate that was studied comes from different batches of fermentation.

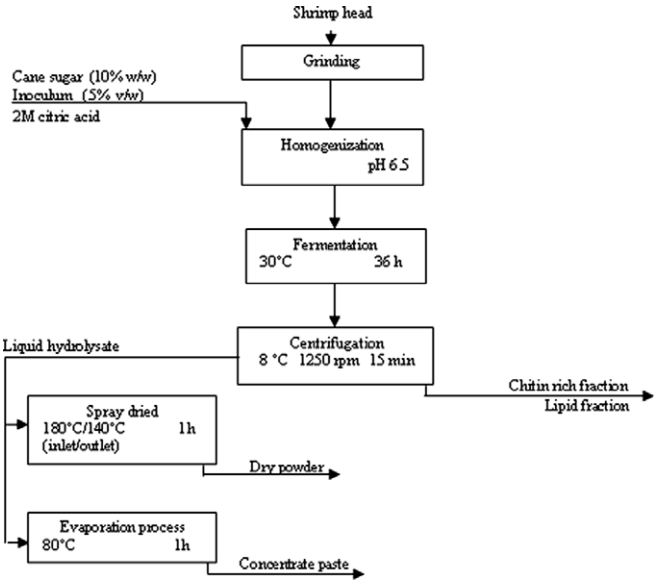


**Fig. 1.** Flow sheet of the process for protein recovery from protein hydrolysates.

The analyzed samples were dry powder, concentrated paste and liquid protein hydrolysate. The samples were stored in amber bottles and were kept in the dark until use.

In producing of the dry powder, the liquid hydrolysate, rich in protein, was dehydrated using a spray dryer SD-04 Lab Scale Spry Drier (LabPlant, Huddersfield, West Yorkshire, England). The liquid hydrolysate was transferred to a conical flask and placed in an electric grill heated to a constant 80 °C. The temperature of the air inlet was 180 °C and the air outlet was 140 °C. The speed of the peristaltic pump was minimized to produce a slow flow of fluid input (1 l/h); the flow of air in the chamber was 100%. The dry sample was collected in glass bottles with lids. The concentrated paste was prepared in a design level laboratory, which consisted of two moisture retaining traps, prepared with silica and cotton. Both were connected to a pump to generate a vacuum. An 800 ml sample was placed in a conical flask to be heated in an electric grill at (80 °C) which will reduce the volume by half in about 1 h.

### 2.3. Quantification of total amino acids

#### 2.3.1. Protein hydrolysis

The conditions used for hydrolysis are modified from those proposed by Sánchez-Machado, López-Cervantes, López-Hernández, Paseiro-Losada, and Simal-Lozano (2003). It is worth mentioning that tryptophan was not determined as it is destroyed during acid hydrolysis. Additionally, methionine and cysteine values may not be accurate due to lack of any special treatment during the hydrolysis. Each sample (50 mg) was placed in tubes and hydrochloric acid (6 M, 10 ml) was added. The tubes were closed under nitrogen, placed in an electric oven at 110 °C for 24 h, cooled, and the contents were vacuum-filtered through Whatman No. 41. The filtrate was diluted to a concentration of 0.02 mg/ml for the dry powder and concentrated paste; while the liquor was diluted to a concentration of 0.2 mg/ml. Samples of hydrolysate (300 μl) were placed in a tube and dried in a vacuum oven for 6 h at 110 °C. The residues were then dissolved in a borate buffer (300 μl) to prepare the solution for the derivatization process.