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

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## ARTICLE INFO

## 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 $8.43 \pm 0.22$ to $46.73 \pm 1.29$ and $2.03 \pm 0.52$ to $8.25 \pm 0.14$ 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

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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/v) 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 pro-
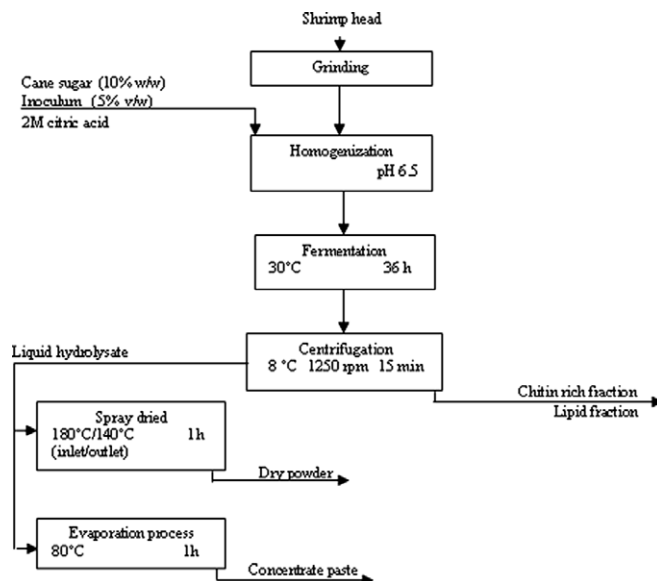


**Fig. 1.** Flow sheet of the process for protein recovery from protein hydrolysates.

tein hydrolysate, and the lipid fraction, Fig. 1. The liquid protein hydrolysate that was studied comes from different batches of fermentation.

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.

### 2.3.2. Derivatization

The method of analysis of López-Cervantes et al. (2006) was followed with minor modifications. To derivatize the solution, 300 μl of prepared sample was deposited in a vial, then 300 μl of FMOC reagent was added and vortexed for 90 s. The cleavage reagent was added (180 μl) and the vial was vortexed for 15 s, then left for 5 min at room temperature. 420 μl of quench reagent was then added. The resulting solution was vortexed for 15 s and filtered with a membrane 0.45 μm. A 5 μl sample of this solution was injected into the column of the HPLC system.

### 2.3.3. Equipment

The HPLC system (GBC, Dandenong, Australia) was equipped with an auto injector LC 1650, an on line solvent degasser LC 1460, a system controller with WinChrom for chromatography data analysis, a pump LC 1150, a column oven LC 1150, a 20 μl injection loop (Rheodyne, Cotati, CA, USA) and fluorescence detector LC 1255 s. The chromatographic analysis was performed using an analytical scale (4.6 mm × 150 mm) Zorbax Eclipse AAA C18 column with particle size 5 μm (Thermo Electron Corporation, Dandenong, Australia). The HPLC conditions are shown in Table 1.

### 2.4. Proximate composition

For moisture content determination, the samples were heated overnight in an electric oven at 60 °C. For ash content, ground dried samples were heated for 5 h in an electric oven at 525 °C. Total protein content was calculated by multiplying Kjeldahl nitrogen by 6.25 (AOAC, 1995).

### 2.5. Special analyses for the dry powder protein hydrolysate

#### 2.5.1. Colour

The measurement of the colour of dry powder samples was determined with a colorimeter; model Chroma Meter 410 (Konica Minolta Holdings, Tokyo, Japan). The colorimeter determined lightness ($L^*$), redness ($a^*$, ±red–green) and yellowness ($b^*$, ±yellow–blue) (Commission Internationale d́Eclairage).

#### 2.5.2. Microbiological analysis

Microbiological analyses were performed according to the AOAC methods (1995), 46.030, 46.062, 46.115, 46.011, and 4.030 for coliforms and Escherichia coli; Staphylococcus aureus; Salmonella; fungi and Pseudomonas, respectively.

#### 2.5.3. Heavy metals

The quantification of Zn, Cu, Fe, Mn, Cd, Pb, and As were previously performed as a wet digestion by the Denton and Burdon–Jones method (1986), while the determination of Hg was performed by the method of Stepson (1985). The analysis was carried out by atomic absorption spectroscopy in a Varian Spectra AA 220 with a UV VGA77 detector (Varian, Melbourne, Victoria, Australia).

### 2.6. Statistical analysis

All data presented are means ± standard deviations. The statistical significance of differences between means ($p < 0.05$) was estimated by Student́s t-tests using SPSS 12.0.

## 3. Results and discussion

### 3.1. Chemical composition

The moisture content of the dry powder, concentrated paste and liquid hydrolysate were 5.78, 32.06 and 83.29%, respectively. The moisture content is related to the kind of sample and to the higher temperatures employed during the process of evaporation and spray drying. In these processes the sample lost most of its moisture.

The moisture content in the concentrated paste research was higher than the 24.39% reported by Synowiecki and Al-Khateeb (2000), where researchers conducted an enzymatic hydrolysis of shrimp by-products. In this test, the liquid hydrolysate moisture content is higher than the 66.9% reported by Fabgenro and Bello-Olusoji (1997), where hydrolysate was concentrated under conditions simulating the drying sun.

Table 2 presents the proximate analysis (wet weight) of the different treatments applied to the hydrolysate. The ash content is in the range of 2.03–8.25%. The dry powder has a higher content of minerals, when compared to the liquid hydrolysate. The dry powder presents a mineral content of 8.25%. The high mineral content can be attributed to the fact that during the lactic fermentation of shrimp by-products, a demineralization occurs. Most of the minerals may be in the hydrolysate, as reported by Jung et al. (2005). The ash content in the concentrated paste is similar to that reported by Ruttanapornvareesakul et al. (2006), while the dry powder was similar to that reported by other researchers (Chakrabarty, 2002; Gildberg & Stenberg, 2001). The ash content in the three treatments was lower than those reported (Duarte de Holanda & Netto, 2006; Fabgenro & Bello-Olusoji, 1997; Nwanna, 2003).

The protein content in the diverse samples was higher than those of Synowiecki and Al-Khateeb (2000) who reported a content of 40.6%. Gildberg and Stenberg (2001) obtained a content of 45.1%. In both reports, researchers used an enzymatic method on the shrimp by-products. Fabgenro and Bello-Olusoji (1997), reported a crude protein content of 41.8% in a hydrolysate from a fermentation of shrimp by-products. Abdul-Hamid et al. (2002) employed a spray drying system for the tilapia hydrolysate, which measured

**Table 1**
Conditions of chromatographic analysis

| Parameters | Condition |
|---|---|
| Mobile phases | A: 30 mM ammonium phosphate (pH 6.5) in 15:85 (v/v) methanol:water; B: 15:85 (v/v) methanol:water; C: 90:10 (v/v) acetonitrile:water |
| Gradient program | (Min/A%:B%:C%): 0/8:79:13, 34/11:43:46, 36/11:43:46, 36.5/0:0:100, 38.5/0:0:100, 38.55/8:79:13, 50/8:79:13. |
| Flow rate | 0.5 ml/min |
| Detection | $\lambda_{ex}$: 270 nm and $\lambda_{em}$: 316 nm |
| Temperature | 38 °C |
| Time of analysis | 50 min |
| Injection volume | 5 μl |

**Table 2**
Protein and ash content in hydrolysates (g per 100 g wet weight)

| Sample[a] | Ash (%) | Average ash | Protein (%) | Average protein |
|---|---|---|---|---|
| *Dry powder* | | | | |
| Batch 1 | 8.18 | | 45.11 | |
| Batch 2 | 8.39 | 8.25 ± 0.14 | 47.61 | 46.73 ± 1.29 |
| Batch 3 | 8.20 | | 47.47 | |
| *Concentrated paste* | | | | |
| Batch 1 | 5.56 | | 28.81 | |
| Batch 2 | 5.54 | 5.52 ± 0.14 | 27.16 | 28.02 ± 1.32 |
| Batch 3 | 5.45 | | 28.11 | |
| *Liquid hydrolysate* | | | | |
| Batch 1 | 1.61 | | 8.41 | |
| Batch 2 | 2.68 | 2.03 ± 0.52 | 8.56 | 8.43 ± 0.22 |
| Batch 3 | 1.79 | | 8.33 | |

[a] Means values of $n = 3$, duplicate determinations ± standard deviations.

49.6% protein. The observed differences are attributable to the kind of the shrimp and the type of recovery process.

## 3.2. Amino acid profile present in the hydrolysate

The nutritional value of a food depends on the type and amount of amino acids available for body functions. Table 3 shows the total amino acid profile of the three different hydrolysates. The total content of amino acids in the powder, in the concentrated paste and in the untreated liquor was 344.8 mg/g of dry weight, 360.6 mg/g of dry weight, and 237.7 mg/g of dry weight, respectively. In both dry powder and concentrated paste forms, tyrosine was the amino acid present in superior quantity at 19.3 and 29.5%, respectively. As for the untreated liquor, alanine was the most abundant amino acid measuring 14.4%. The total essential amino acids present in the liquid hydrolysate, dry powder and concentrated paste were 47.0, 61.4 and 53.1%, respectively. The higher concentration of histidine was 3.3% (liquid hydrolysate), arginine 6.4% (dry powder), threonine 4.7% (liquid hydrolysate), tyrosine 29.5% (concentrated paste), valine 7.1% (liquid hydrolysate), methionine 5.0% (concentrated paste), isoleucine 5.6% (concentrated paste), leucine 6.8% (liquid hydrolysate) and phenylalanine 5.7% (liquid hydrolysate). The histidine was the limiting essential amino acid in the dry powder and concentrated paste, while for the liquor was methionine. The essential amino acid of higher concentration in the three samples was tyrosine. Due to the high temperatures subjected to the hydrolysate, both in the process of spray drying and in the formation of a paste, there may be a loss of a portion of the amino acids in the samples (Abdul-Hamid et al., 2002). In non-essential quantified amino acids, the highest concentration in the dry powder was the glutamic acid (38.8 mg/g dry weight), while alanine had the highest concentration both in the concentrated paste (44.1 mg/g dry weight) and the liquid hydrolysate (34.1 mg/g dry weight). The amino acid in lower concentrations in the three hydrolysates was serine, with concentrations of 4.6, 9.8, and 10.8 mg/g dry weight in dry powder, concentrated paste and liquid hydrolysate, respectively.

The concentrations obtained were slightly lower than those reported in other research, where the shrimp by-products were treated with an enzyme hydrolysis. Such is the case of Ruttanapornvareesakul et al. (2006), who found that the amino acid in higher concentration was glutamic acid and the limiting amino acid was methionine. Gildberg and Stenberg (2001) and Shahidi and Synowiecki (1991), reported histidine as a limiting essential amino acid and leucine as the biggest concentration, in both cases,

the proteins were removed from shrimp by-products. Mizani et al. (2005), pointed to histidine as the limiting amino acid, and threonine as the more concentrated.

In previous research, where fermentations were carried out to treat shrimp by-products, similar values of the essential and non-essential amino acids were found. Nwanna (2003) reports the amino acid, phenylalanine, to be in greater concentration; Nwanna et al. (2004), recorded valine as having the highest concentration. Toma and Meyers (1975), recorded that glutamic acid was the amino acid in higher concentration. López-Cervantes et al. (2006) reported a total concentration of free amino acids in lyophilized liquor, similar to concentrated paste.

## 3.3. Special analyses for dry protein powder hydrolysate

### 3.3.1. Determination of colour

Colour has a major influence on the presentation value of a product. The dry powder protein hydrolysate has a brown colour, showed low values for brightness ($L^*$), as in $b^*$, however; $b^*$ values are greater than $a^*$. In Table 4 the values obtained are shown. Brown colour can be defined as slight light yellow. The brown colour of dry powder is possibly due to the components that were generated in the Maillard reaction during the drying period (Lario et al., 2004).

Colour contributes to measuring the quality of a food, which attracts the consumer. Colour comes from different sources, sometimes colour is determined during handling or processing. The caramelizing and synthesizing of pigments or the fermenting processes can modify colours. Factors such as light, oxygen, temperature and water may affect colour stability.

### 3.3.2. Microbiological determination

The presence of certain microorganisms in a food product indicates the consumption safety of the product. The results of microbiological analyses from dry powder were negative for coliforms, E. coli, Staphylococcus aureus, Salmonella, fungi and Pseudomonas. On the other hand, the total mesophilic count was 1730 colony forming units (CFU) per g, however; this level is not a health risk. The results comply with standard specifications for the consumption of fresh shrimp NOM-027-SSA1-1993 (FAOLEX, 1994). Singh, Chan, Ng, and Yong (1987), analyzed freshwater shrimp (shell-on), with a count of 82 and 14 most probable number (MPN) per g of coliforms and E. coli, respectively. Ninety-nine percent of the sample had less than 50 MPN per g of S. aureus, and Salmonella tested positive in three samples. While Valdimarsson, Einarsson, Gudbjörnsdottir, and Magnusson (1998), analyzed, through a shrimp processor, the result of 1728 aerobic plate count (APC) per g, 70% of the sample had less than one coliform per g, and 99% of the sample had less than one fecal coliform per g. S. areus was detected in less than 0.2% of the sample. In the dry powder form of shrimp by-products, pathogenic microorganisms were not detected. The zero content of pathogenic bacteria and the low presence of mesophilic microorganisms in the dry powder, gives a high hygiene value that can be consumed without presenting a health risk.

### 3.3.3. Determination of metals in dry powder protein hydrolysate

High concentrations of heavy metals are a risk to the consumers' health. The presence of heavy metals in some products of mar-

**Table 3**
Amino acid composition of protein hydrolysates (mg per g dry weight)[a]

| Amino acid | Dry powder | Concentrated paste | Liquid hydrolysate |
|---|---|---|---|
| Asp | 34.6 ± 5.5 | 23.5 ± 5.0 | 14.8 ± 13.4 |
| Glu | 38.8 ± 5.9 | 18.5 ± 3.8 | 26.9 ± 6.9 |
| Ser | 4.6 ± 1.6 | 9.8 ± 10.9 | 10.8 ± 4.6 |
| His | 9.0 ± 3.2 | 7.4 ± 6.2 | 7.8 ± 0.7 |
| Gly | 21.8 ± 1.2 | 19.9 ± 0.2 | 24.9 ± 4.4 |
| Thr | 12.1 ± 1.1 | 13.7 ± 1.8 | 11.2 ± 3.7 |
| Ala | 36.1 ± 5.4 | 44.1 ± 4.3 | 34.1 ± 0.6 |
| Pro | 25.8 ± 7.7 | 23.5 ± 5.1 | 14.6 ± 4.2 |
| Tyr | 66.4 ± 12.2 | 106.4 ± 22.1 | 24.0 ± 18 |
| Arg | 22.2 ± 1.3 | 12.5 ± 2.7 | 7.0 ± 1.5 |
| Val | 16.9 ± 4.0 | 13.0 ± 2.4 | 17.0 ± 1.4 |
| Met | 16.4 ± 10.4 | 17.9 ± 5.7 | 2.6 ± 0.3 |
| Ile | 18.3 ± 6.3 | 20.1 ± 6.0 | 12.2 ± 1.3 |
| Leu | 11.8 ± 7.8 | 20.0 ± 7.6 | 16.2 ± 0.9 |
| Phe | 10.0 ± 1.4 | 10.1 ± 1.4 | 13.6 ± 1.1 |
| $\sum$ Amino acid total | 344.8 | 360.6 | 237.7 |
| $\sum$ Essential amino acid | 183.1 | 221.2 | 111.6 |

[a] Means values and standard derivations (±), n = 3, duplicate.

**Table 4**
$L^*$, $a^*$ and $b^*$ from dry powder protein hydrolysate

| Sample[a] | $L^*$ | Average | $a^*$ | Average | $b^*$ | Average |
|---|---|---|---|---|---|---|
| Barch 1 | 46.93 | | 11.62 | | 18.66 | |
| Batch 2 | 44.99 | 44.42 ± 2.67 | 12.89 | 11.93 ± 0.84 | 19.60 | 17.86 ± 2.25 |
| Batch 3 | 41.65 | | 11.28 | | 15.31 | |

[a] Means values of n = 3, duplicate (dw) determinations ± standard deviations.

**Table 5**
Concentration of heavy metals in the from dry powder protein hydrolysate

| Metals | Concentration |
| --- | --- |
| Zn (mg/Kg) | 78.28 |
| Cu (mg/Kg) | 49.20 |
| Fe (mg/Kg) | 124.30 |
| Mn (mg/Kg) | 13.30 |
| Cd (mg/Kg) | ⩽0.002 |
| Pb (mg/Kg) | ⩽0.01 |
| As (µg/Kg) | ⩽0.20 |
| Hg (µg/Kg) | 1.00 |

ine origin can be traced to increased water pollution. Quantities of heavy metals in dry powder are recorded at levels below the standard, in reference to WHO standards. The concentration of heavy metals that were identified in the dry powder is presented in Table 5. These values are similar to those reported by Paéz-Osuna and Tron-Meyer (1996), who analyzed exoskeleton of bay shrimp and found values of 45.9 (µg metal per g dry weight.) for Cu, 33.2 (µg metal per g dry weight) for Mn, 1.0 (µg metal per g dry weight) for Ni, 66 (µg metal per g dry weight) for Fe and 23 (µg metal per g dry weight) of Zn. There was no measurable concentration of Cd. In analyzing the muscle of male and female shrimp, Yılmaz and Yılmaz (2007), identified concentrations of silver at 2.1–2.8 (µg metal per g wet weight), chromium at 5.9–6.8 (µg metal per g wet weight), nickel at 0.6–3.0 (µg metal per g wet weight), lead at 0.6–0.6 (µg metal per g wet weight), copper at 25.4–25.7 (µg metal per g wet weight), iron at 10.7–12.5 (µg metal/per g wet weight) and zinc at 4.3–6.0 (µg metal per g wet weight), respectively. Heu, Kim, and Shahidi (2003) identified quantities of metals in the by-products of shrimp (heads, shells, and tails) as: copper (7.4 ppm), zinc (13.9 ppm), cadmium (0.3 ppm), mercury (trace) and lead (0.3 ppm).

## 4. Conclusions

The liquid hydrolysate, concentrated paste and dry powder containing a high content of essential amino acids and ash, suggest that the protein hydrolysates, derived from the lactic fermentation of shrimp by-products, have a high nutritional content, as well as a low metal and microorganism content. Spray drying is a novel technique that can concentrate the protein content. Also, the powder has a lower moisture content which extends the storage life and reduces the microbial flora. Spray drying also facilitates transport. The products obtained from processing shrimp by-products can be incorporated as high value supplements in human and animal diets.

### Acknowledgements

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