DETERMINATION OF THE CHEMICAL COMPOSITION AND FUNCTIONAL PROPERTIES OF SHRIMP WASTE PROTEIN CONCENTRATE AND LYOPHILIZED FLOUR

Determinação da composição química e das propriedades funcionais de concentrado protéico e de farinha liofilizada de resíduos de camarão

Olivaldo Lacerda Brasileiro[1], José Marcelino Oliveira Cavalheiro[2], João Paulo de Sousa Prado[2], Alinne Gouveia dos Anjos[2], Thais Tereza Brandão Cavalheiri[3]

ABSTRACT

Wastes from the seafood industry can be easily processed into products with new forms of use. The present study was aimed at determining the chemical composition and functional properties of shrimp waste protein concentrate and lyophilized flour. The raw material used in this study consisted of waste (head) of *Litopenaeus vannamei*. The protein concentrate was obtained by ethanolic extraction, being subsequently submitted to drying in oven at 70° C, while the flour was obtained by lyophilization of shrimp wastes. Moisture, ash, protein and calcium contents showed significant difference between fresh shrimp head waste samples (IN) and protein concentrate and lyophilized flour samples. The protein content for protein concentrate (PC) and lyophilized flour (LF) showed significant increase in relation to protein content in the waste (IN), thus showing that the process for obtaining the protein concentrate was efficient.

Index terms: *Litopenaeus vannamei*, food source, environment, solubility, pH.

RESUMO

Resíduos provenientes da indústria do pescado podem ser facilmente transformados em produtos com novas formas de aproveitamento. No presente estudo, objetivou-se determinar a composição química e as propriedades funcionais de farinha liofilizada e concentrado proteico provenientes de resíduos de camarão. A matéria-prima empregada neste estudo foi constituída de resíduos (cabeça) de camarão *Litopenaeus vannamei.* O concentrado proteico foi obtido por extração etanólica e, posteriormente, submetido à secagem em estufa a 70° C, enquanto a farinha foi obtida pelo processo de liofilização do resíduo. Os teores de umidade, cinzas, proteínas e cálcio apresentaram diferença significativa entre as amostras de resíduo de cabeça de camarão *in natura* (IN) e as amostras de concentrado proteico e farinha liofilizada. O teor de proteínas para concentrado protéico (CP) e para a farinha liofilizada (FL) apresentou aumento significativo em relação ao teor proteico do resíduo (IN), mostrando, assim, que o processo de elaboração do concentrado proteico foi eficiente.

Termos para indexação: *Litopenaeus vannamei*, fonte alimentar, meio ambiente, solubilidade, pH.

(Received in march 24, 2012 and approved in april 30, 2012)

INTRODUCTION

In Brazil, the use of waste in the fish production cycle is negligible; only in the canning industry this residue is used for fishmeal production. Fish processing industry wastes represent a serious problem, mainly because they are pollutants difficult to dispose, interfering with the production process efficiency (GUILHERME et al., 2007).

During shrimp processing, the peeling step generates large amount of solid waste, because head and peel represent approximately 40% of the shrimp weight (GILDBERG; STENBERG, 2001).

Fish protein concentrate has all the characteristics of a food widely used around the world, solving problems of the use of fish and / or its waste. It is a concentrated product containing proteins (75%) with the following basic characteristics: low cost, low fat and moisture contents, deodorized, high digestibility, easy storage, does not require refrigeration and long shelf life. The large capacity of hydration and functional properties facilitate the preparation of various foods (sausage or formulated). The basic technology used in the production of fish protein concentrate is the concentration of its protein through lipid extraction with solvents, using the chemical method (OETTERER, 2011).

Shrimp head flour (SHF) is made from the dehydration of industrial shrimp waste and is basically composed of heads, exoskeletons and small shrimp. In the shrimp processing, 50% of the animal weight result in

[1]Universidade Federal da Paraíba/UFPB – Campus I – s/n – Cidade Universitária – 58051-270 – João Pessoa – PB – Brasil – olivaldolacerda@hotmail.com
[2]Universidade Federal da Paraíba/UFPB – João Pessoa – PB – Brasil
[3]Pontifícia Universidade Católica do Rio Grande do Sul/PUC-RS – Porto Alegre – RS – Brasil

by-products during industrialization. Only the head is responsible for about 44% of this waste (GERNAT, 2001).

The present study was aimed at determining the proximate composition and functional properties of shrimp waste protein concentrate and lyophilized flour.

MATERIAL AND METHODS

The raw material used in this study consisted of waste (head) of *Litopenaeus vannamei* from farm in the coastal region of João Pessoa - PB and transported to LDPP (Laboratory of Fishery Product Development), Technology Center - CT – UFPB, which has been processed to obtain protein concentrate and lyophilized flour.

The samples were washed with distilled water at approximately three times the volume of the sample for 5 minutes, repeating the procedure three times. The washing time depends on the amount of fatty matter in the sample, and in this case, a 20-minute rest time was applied. Excess water was removed using a strainer and filter paper, and the pH was adjusted to 7.4 - 7.8, with 1% sodium chloride. After removal of the excess distilled water, the sample was placed in a beaker with cooled ethanol (5° C-10° C), and the ethanol volume was equivalent to three times the sample volume, kept under shaking for 20 minutes and centrifuged, (3400rpm - CT, 5000 – R- cientec – Brazil) repeating this procedure twice. Excess ethanol was removed by filtration. Then, the residue was dried in stove at 70° C. Finally, the dried residue was crushed and sifted to obtain the protein concentrate (QUAGLIA; ORBAN, 1987).

After washing, the samples were submitted to immersion in boiling water for 15 minutes and allowed to cool to room temperature, then submitted to freezing process (-20° C) and finally lyophilization (Lyophilizer LS3000 - Terroni) for a period of 16 hours. The lyophilized residue was crushed in industrial mill (MA340 – Marconi – Brazil) and sifted to obtain the flour.

To characterize the protein concentrate, the methods described by the analytical standards of the "Adolfo Lutz" Institute (SAO PAULO, 2008) were used, for the following analysis: moisture by desiccation in oven at 105°C to constant weight, ash by incineration in a muffle furnace at 550° C, calcium by titration, lipid by the soxhlet method and total protein by the Kjeldahl method (N x 6.25).

Solubility was determined according the method of Dench et al. (1981), in which 0.1 g of protein concentrate and flour was weighed in centrifuge 3400rpm tubes, added of 10 ml of distilled water, shaking the suspension for 30 seconds in a tube shaker and taken to water bath with thermostat (THERMOMIX BM - B. Braun Biotech Internacional) for a period of 30 minutes, with temperature ranging from 55° C to 95° C. Then, the tubes were removed from the bath and cooled to room temperature, centrifuged at 3400 rpm / 20 min (CT, 5000 – R- CIENTEC – Brazil), 5 ml of supernatant were collected and placed in test tubes and dried to constant weight at 105° C to determine the solubility.

The water and oil absorption capacity was determined according to the method described by Beuchat (1977). About 10.0 ml of distilled water or oil were added to 1 g of concentrate in previously weighed centrifuge tubes. The suspension was shaken in tube shaker for 30 seconds. The suspension remained at rest for 30 minutes and then centrifuged at 2000 rpm / 15 min (CT, 5000 – R- cientec – Brazil). The supernatant was discarded, and tubes were inverted to drain the oil / water for 10 min. The tubes were weighed on an analytical balance (edutec – EEQ9009 – Brazil) again and the weight gain was used to determine the oil and water absorption capacity in g / 100g (dry basis).

The results of the analysis, in triplicate, were submitted to the Kolmogorov-Sminorv (KS) statistical test in order to assess the distribution normality, followed by Statistical Analysis of Variance (ANOVA) and multiple comparisons performed by the Tukey test. Level of error probability (p) lower than 0.05 or 5% was considered to define the significance of variance tests, which together with means and standard deviations, were performed using the SPSS for Windows software - 11.0 (SPSS. INC, 2001), according to Marocco (2007).

RESULTS AND DISCUSSION

Moisture, calcium, lipids, proteins and ash contents (IN) are represented as shown in table 1.

Table 1 – Chemical composition of fresh shrimp head (IN) protein concentrate (PC) and lyophilized flour (LF) on a dry basis.

	MOISTURE	CALCIUM	LIPIDS	PROTEIN	ASH
IN	78.60^{a}±0.95	3.16^{c}±0.16	0.88^{b}±0.07	12.43^{b}±0.65	6.59^{c}±1.12
PC	7.49^{b}±0.04	6.48^{a}±0.09	1.16^{b}±0.14	54.41^{a}±3.10	19.19^{a}±1.52
LF	4.99^{c}±0.04	6.14^{b}±0.04	3.56^{a}±0.35	51.01^{a}±1.28	15.75^{b}±0.91

Different lowercase letters in the same column differ significantly at 5% by the Tukey test.

Gonçalves and Ribeiro (2009) for white shrimp and Furuya et al (2006) for freshwater prawn (*Macrobrachium amazonicum*), found moisture (75.8 and 70,3%) and ash values (1.65 and 1,5%), respectively, slightly below those found in this study for white shrimp (*Litopenaeus vannamei*). However, these authors found higher values for lipids (2.51 to 1,5%) and protein (15.84 and 24,7%), respectively.

Moisture, ash, protein and calcium contents showed a significant difference between fresh shrimp head waste samples (IN) and protein concentrate and lyophilized flour samples.

The protein content for protein concentrate (PC) showed a significant increase in relation to the protein content in the waste (IN), thus showing that the process of preparing the protein concentrate and lyophilized flour was effective, with no significant difference between them. According to Pessatti (2001), the protein concentration should be an average of four times the protein content value of the raw material; therefore, the method use to produce PC was efficient. Murueta et al. (2007) studied various drying methods in the production of protein concentrate of nine fish species and found that the protein content ranged from 57% and 77%, with no difference between different drying methods applied (lyophilization , drying at 65° C for 15 hours drying at 100° C for 12 h).

The moisture content in the waste showed a significant reduction when submitted to the process of preparing the protein concentrate and lyophilized flour, and the lyophilized flour showed the lowest moisture content. The dehydration of the protein concentrate in this work could be considered very efficient, since Ogawa (1999) reported that the muscle of fresh fish can contain 60-85% of moisture content. Vila Nova et al. (2005) characterized the chemical composition of tilapia fillets (*O.niloticus*) and reported 77.55% of moisture content.

Regarding ash and calcium, the protein concentrate had the highest value compared to the fresh waste and lyophilized flour, pointing out that the difference between protein concentrate and lyophilized flour was small. Comparing the results of lyophilized flour obtained from waste with those obtained from fresh shrimp waste, it was found that the lyophilized flour showed ash values 2 times higher than values obtained for fresh waste. Freitas et al (2002) studied shrimp waste flour and found values 4 times higher than for fresh shrimp. According to Wang and Hwang (2001), the composition of minerals consists mainly of calcium carbonate, phosphate, magnesium, silicon and sulfur. This increased ash content, primarily with regard to calcium, indicates potential use of this flour in diets for special purposes.

The content of lipids in the lyophilized flour (LF) showed a significant increase compared to the lipid content of the fresh waste (IN) and protein concentrate (PC) was observed, since the lyophilization process promotes the concentration of chemical components.

In the case of the lipid content, a variation between waste and lyophilized flour was observed. It could be observed that there was an increase in the percentage of proteins and a decrease in the percentage of lipids, when CMS and protein concentrate were compared. The protein content found in the protein concentrate was almost twice that of CMS and lipids showed values nine times lower in the protein concentrate, when compared to CMS. Moisture, which is a critical factor in the processing of surimi, showed values consistent with those observed by other authors who also use CMS as raw material for obtaining protein concentrate (90.1%, SMYTH and O'NEILL, 1997; 89.5%, CORTEZ-VEGA *et al*., 2008). However, it would be desirable to obtain lower moisture values, since in surimi-based products, moisture will form a gel with better quality, which reflects in a higher gel strength.

It was found that the solubility of the protein concentrate was not significantly different ($p < 0.05$) in temperature ranging from 65° C to 95° C. However, in the case of lyophilized flour, the lowest solubility value was found at temperature of 85° C. In the temperature range studied, the solubility values for lyophilized flour were above those of the protein concentrate (Figure 1).

It was found that the solubility of protein concentrates and lyophilized flour showed no significant difference ($p < 0.05$) in pH values from 2.0 to 7.0.

According to Sgarbieri (1996), the isoelectric point (pI) of proteins can vary between 4.0 and 6.0; therefore, there is no value representing the isoelectric point the protein concentrate and lyophilized flour in the range established for this study where the number of positive and negative charges tend to neutralize intramolecularly, with lower affinity for the solvent. Sathivel (2003) studied the influence of pH on the solubility of protein isolates from different fish species and found lower solubility values at pH close to 4.0. In the present study, although there was no statistical difference between protein concentrate and lyophilized flour, it could be observed that in the case of protein concentrate, the lowest solubility value was found at pH 2.0. In the case of lyophilized flour, the lowest value was obtained at pH 5.0.

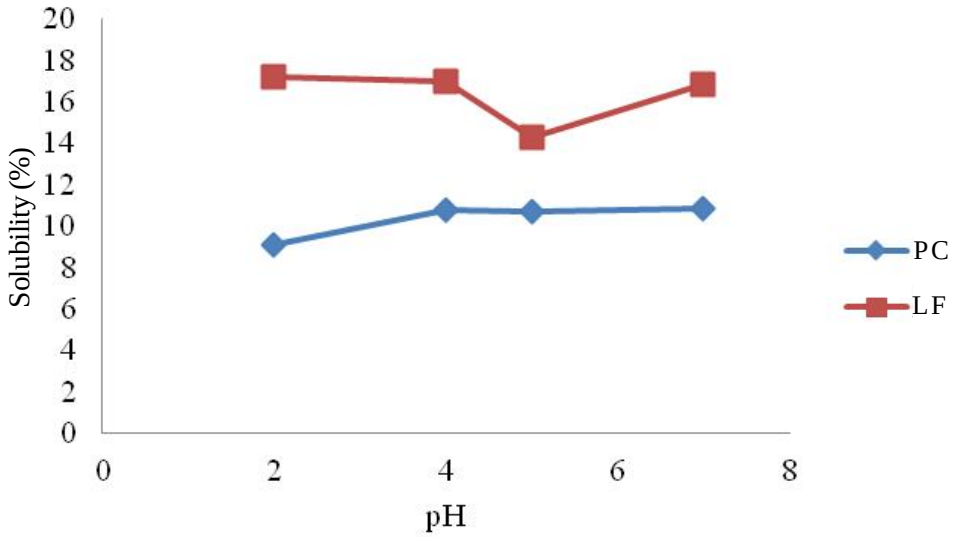

Figure 1 – Solubility of protein concentrate (PC) and lyophilized flour (LF) as function of pH.

Increased solubility in short pH ranges was not obtained as studied by other authors. This increase has been observed for various types of chemical modifications, which introduce negative charges on the protein surface. Studies performed with wheat flour protein, myosin, soybeans, fish and yeast showed the same solubility behavior for values below the isoelectric point (KINSELLA, 1987). This could be due to different buffering capacities of amino acids in protein molecules (KIM, PARK, CHOI, 2003).

Solubility is one of the most important features of proteins and varies considerably in function of pH and ionic strength. Most food proteins exhibit U-shape protein solubility curve in function of pH. Unlike studies on other fish species (sardine, catfish and croaker), there was no isoelectric point for protein concentrate and lyophilized flour in the pH range from 2.0 to 7.0. Batista et al. (2007); Kristinsson et al, 2005; Martins et al, 2009 and Fontana (2009) found higher solubility values at the extremes of the pH range from 3.0 to 11.0, and the isoelectric point was obtained at pH 5.0.

In this study, the average OAC values obtained for protein concentrate and lyophilized flour were 30.23 and 33.40g/100g, respectively. Table 2 shows the mean OAC values to protein concentrate and lyophilized flour obtained by ethanol solubilization.

In this study, the average WAC and OAC obtained for shrimp waste protein concentrate and lyophilized flour showed no significant difference.

Table 2 – Water and oil absorption capacity of protein concentrate and lyophilized flour.

Product	WAC (g/100g)	OAC (g/100g)
Protein Concentrate	35.05^{a}±0.38	30.23^{a}±0.76
Lyophilized Flour	35.53^{a}±3.76	33.40^{a}±4.20

WAC - Water absorption capacity. - OAC - Oil absorption capacity. Different lowercase letters in the same column differ significantly at 5% by the Tukey test.

The oil absorption capacity is one of the most important functional properties in the preparation of products, and can influence the order of addition of dry ingredients in the mixture, thus contributing to a uniform distribution (FONTANA, 2009). According to Kinsella (1987), high OAC values are desirable in products such as meat extenders to improve its mouthfeel and viscous products such as soups, processed cheeses and pasta.

The oil retention mechanism is mainly due to the physical capture of the oil by protein and is a very important functional feature required by the food industry of meats and emulsified products (BOBBIO; BOBBIO, 1995); (SATHIVEL; BECHTEL, 2006).

The oil retention capacity varies depending on the number of protein-exposed hydrophobic groups, and probably, non-polar side chains of proteins have affinity for the hydrophobic chains of the oil molecule, contributing to absorption and improvement of the ORC (KINSELLA, 1982); (DONADEL; PRUDENCIO-

FERREIRA, 1999). Fontana (2009) found for concentrates obtained by alkaline and acid processes, ORC values of 4.7 and 4.6 ml oil / g protein, respectively.

CONCLUSION

It was concluded that products obtained from shrimp waste (protein concentrate and lyophilized flour) can be used in the formulation of ingredients or food products with high protein content aimed at humans and animals, showing physicochemical and functional parameters adequate to characterize them as a good-quality protein supplement.

REFERENCES

BATISTA, I.; PIRES, C.; NELHAS, R. Extraction of Sardine Proteins by Acidic and Alkaline Solubilisation. **Food Science and Technology International**, Los Angeles, v.13, n.3, p.189-194, jun. 2007.

BEUCHAT, L.R. Functional and eletrophoretic characteristics of succinylated peanut flour protein. **Journal of Agricultural and Food Chemistry**, v.25, n.2, p.258-265, mar. 1977.

BOBBIO, F.; BOBBIO, P.A. **Introdução à química de alimentos**. São Paulo: segunda edição, Editora Varela: 1995.

CORTEZ-VEGA, W. et al. 2008. **Influence of the process parameters on the characteristics of chicken surimi**. In: Tenth International Congress on Engineering and Food, Viña del Mar. Tenth International Congress on Engineering and Food, p. J11.

DENCH, J.E.; RIVAS, R.N.; CAYGILL, J.C. Selected functional properties of sesame (*Sesamum indicum* L.) flour and two protein isolates. **Journal of the Science of Food and Agriculture**, London, v.32, n.6, p.557-564, jun. 1981.

DONADEL, M.E.; PRUDENCIO-FERREIRA, S.H. Functional properties of aged bean protein concentrate. **Ciência e Tecnologia de Alimentos**, Campinas, v.19, n.3, p.380, set./dez. 1999.

FONTANA, A. et al. Obtenção e avaliação de concentrados protéicos de corvina (*Micropogonias furnieri*) processados por extração química. **Química Nova**, São Paulo, v. 32, n.9, p.2299-2303, out. 2009.

FREITAS, A.S. et al. Composição química e protéico-molecular da farinha de resíduos de camarão-sete-barbas (*Xiphopenaeus kroyeri*). **B. CEPPA**, Curitiba, v.20, n.1, p.111- 120, jan./jun. 2002.

FURUYA, W. M. et al. Composição centesimal e perfil de ácidos graxos do camarão-d'água-doce. **Revista Brasileira de Zootecnia,** v.35, n.4, p.1577-1580, ago. 2006.

GERNAT, A. G. The effect of using different levels of shrimp meal in laying hen diets. **Poultry Science**, Champaign, v.80, n.5, p.633-636, maio. 2001.

GILDBERG, A.; STENBERG, E. A new process for advanced utilization of shrimp waste. **Process Biochemistry**, Amsterdam, v.36, n. 8-9, p.809 812, março. 2001.

GONÇALVES, A.A.; RIBEIRO, J.L.D. Do phosphates improve the seafood quality? Reality and legislation. **Pan-American Journal of Aquatic Sciences**, v.3, n.3, p.237-247. 2008.

GUILHERME, R.F.; CAVALHEIRO, J.M.O.; SOUZA, P.A.S. Caracterização química e perfil aminoácidico da farinha de silagem de cabeça de camarão. **Ciência e Agrotecnologia**, Lavras, v.31, n.3, p.793-797, mai./jun. 2007.

KIM, Y.S.; PARK, J.W.; CHOI, Y.J. New approaches for the effective recovery of fish proteins and their physicochemical characteristics. **Fisheries Science**, v.69, n.6, p.1231-1239, dezembro. 2003.

KINSELLA, J.E. Relationships between structure and functional properties of food proteins; In: FOX, P. F., CONDON, J. J. **Food proteins** eds.; Applied Science Publishers: London, 1982. p.59-99.

KINSELLA, J.E. In: Knorr, D. **Food Biochemistry**. New York: ed., Marcel Dekker, 1987. cap. 14.

KRISTINSSON, H.G. et al. A Comparative study between acidand alkali-aided processing and surimi processing for the recovery of proteins from channel catfish muscle. **Journal of Food Science,** Malden, v.70, n.4, p. 298-306, maio.2005.

MAROCCO, J. **Análise estatística com utilização do SPSS**. Lisboa: Ed. Silabo, 824p. 2007.

MARTINS, V.G.; COSTA, J.A.V.; PRENTICE-HERNÁNDEZ, C. Hidrolisado protéico de pescado obtido por vias química e enzimática a partir de corvina (*Micropogonias furnieri*). **Química Nova**, São Paulo, v.32, n.1, p.61-66, nov.2009.

MURUETA, J.H.C.; TORO, M de los A.N. del; CARRRENO, F.G. Concentrates of fish protein from bycatch species produced by various drying processes. **Food Chemistry**, v.100, n.2, p.705-711, outubro. 2007.

OETTERER, M. **Proteínas do Pescado.** Escola Superior de Agricultura "Luiz de Queiroz". São Paulo-SP. Disponível em: <http://www.unimep.br/phpg/editora/revistaspdf/rct19art09.pdf> Acesso em: 25 de maio de 2011, às 15:10h.

OGAWA, M. **Química do pescado:** Umidade e Proteína. In: OGAWA, M.; MAIA, E. L. (Eds), Manual de Pesca. v. I: Ciência e Tecnologia. São Paulo: Varela, 1999. Cap. 4, p. 29-48.

PESSATTI, M. L. **Aproveitamento dos subprodutos do pescado**. Meta 11. Relatório Final de Ações Prioritárias ao Desenvolvimento da Pesca e Aqüicultura no Sul do Brasil, Convênio Ministério da Agricultura, Pecuária e Abastecimento (MAPA), Universidade do Vale do Itajaí, MA/SARC, n.003/2000. 2001.

QUAGLIA, G.B., ORBAN, E. Enzymatic solubilisation of proteins of sardine (*Sardina pilchardus*) by commercial proteases. **Journal of the Science Food and Agriculture**, London, v.38, n.3, p.263-269, setembro. 1987.

SÃO PAULO, INSTITUTO ADOLFO LUTZ – Normas Analíticas do Instituto Adolfo Lutz. **Métodos Químicos e Físicos para Análises de Alimentos**. 3 ed., São Paulo, 2008.

SATHIVEL, S. **Annual Meeting Book of Abstracts**, Chicago, USA, 2003.

SATHIVEL, S.; BECHTEL, P.J. Properties of soluble protein powders from Alaska pollock (*Theragra chalcogramma*), **International Journal of Food Science & Technology**. 2006, v.41, n.5, p.520-529, maio.2006.

SGARBIERI, V.C. **Proteínas em Alimentos Protéicos**: Propriedades, Degradações, Modificações, Editora Varela: São Paulo, 520 p., 1996.

SMYTH, A.B.; O'NEILL, E. Heat-induced gelation properties of surimi from mechanically separated chicken. **Journal of Food Science**, v.62 n.2, p.326-330, julho. 1997

VILA NOVA, C.M.V.M.; GODOY, H.T.; ALDRIGUE. M.L. Composição química, teor de colesterol e caracterização dos lipídios totais de tilápia (*Oreochromis niloticus*) e pargo (*Lutjanus purpureus*). **Ciência e Tecnologia de Alimentos**, Campinas, v.25, n.3, p.430-436, agosto. 2005.

WANG, S.L.; HWANG, J.R. Microbial reclamation of shellfish waste for the production of chitinases. **Enzyme and Microbial Technology**, v.28, n.4-5, p.376-382, março. 2001.