Research Article **Biotechnology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

FATTY ACID COMPOSITION AND TOTAL LIPID CONTENT IN PROTEIN HYDROLYSATES OF SHRIMP HEADS

JAIME LÓPEZ-CERVANTES[1], DALIA I. SÁNCHEZ-MACHADO[1*], CAROLINA BUENO-SOLANO[1], OLGA N. CAMPAS-BAYPOLI[1] AND NORMA P. ADAN-BANTE[2]

[1]Departament of Biotechnology and Food Science, Technological Institute of Sonora, 5 de Febrero 818 Sur, Cd. Obregón, MX-85000 Sonora, México
[2]Departament of Chemical-Biological Sciences, University of Sonora, Boulevard Lázaro Cárdenas 100, MX-85880 Navojoa, Sonora, México

ABSTRACT

Lipids, being recognized as an important source of energy, are major constituents of our diets. The by-products of the shrimp industry have high added value, one of these is protein hydrolyzate obtained by lactic fermentation. In this research concerning fermented shrimp heads, three consistencies of hydrolyzed protein were analyzed: liquid, paste, and powder. The lipid content was in a range of 4.33-7.75% for the hydrolyzed liquid, 6.32-9.51 % for the concentrate in paste form and 24.28-36.78% for the hydrolyzate as a powder. Gas chromatography was employed in the determination of the fatty acid composition (%). The fatty acid in highest concentration in the liquid hydrolyzate was eicosapentanoeic acid (C20:5n3), while the paste and the powder hydrolyzate registered was hexadecanoic acid (C16:00). The paste and the powder hydrolyzates, contained a higher concentration saturated fatty acid than unsaturated. This research complements the characterization of the protein hydrolyzate obtained from the fermentation of shrimp heads.

KEYWORDS: **Lipid, shrimp waste, lactic fermentation, gas chromatography**

DALIA I. SÁNCHEZ-MACHADO
Department of Biotechnology and Food Science, Technological Institute of Sonora, 5 de Febrero 818 Sur, Cd. Obregón, MX-85000 Sonora, México

Corresponding author

INTRODUCTION

The term lipid is used to encompass all types of compounds that are soluble in organic solvents and are classified as fats. Lipids play many roles in the body tissue. Besides being an important energy source, many of the lipids are an important factor in biological activity, in the structure of cell membranes, and in the transport of various nutrients[1]. The two major classes of lipids in food are phospholipids and triacyglicerols[2]. Phospholipids are constituents of cell membranes while triacyglicerols are fatty globules that exist primarily as coalesced droplets in biological tissue. Lipids are susceptible to oxidation, which results in the formation of off-odors and off-flavors, and in the loss of nutritional value in protein and vitamin co-oxidation[3]. Fish and other types of marine-derived foods are good sources of long-chain polyunsaturated fatty acids (PUFAs) belonging to the n-3 family, and most marine oils are good sources of eicosapentaenoic acid (EPA) and docosahexanoenoic (DHA)[4]. Highly unsaturated fatty acids (HUFA) such as EPA (20:5n3) and DHA (22:6n3), found in shrimp tissue, are considered to be essential fatty acids[5]. PUFAs and their derivatives are important neutraceutical and pharmaceutical targets[6]. These fatty acids, EPA and DHA, are known to play a major role in several biochemical processes, both in vivo and in vitro[7]. DHA in the diet has a positive effect on preventing and curing several diseases as coronary heart disease, atherosclerosis and some cancers[7, 8].

Total fat and fatty acid determination in food products comprises several steps and, depending on the type of food, laws and analysis procedures, different results should be generated[9]. Diverse methods have been used for the determination and quantification of fatty acids, for example, NIRS technology with fiber-optics[10], gas chromatography[11], HPLC and capillary electrophoresis[12]. Fatty acids are usually analyzed by gas chromatography (GC) in the corresponding fatty acid methyl esters (FAME). The fatty acids receiving the most focus are usually bonded as esters in large molecules in the sample matrix. The preparation of FAME involves the extraction of the lipid molecules from the sample matrix, breaking of the ester bonds, and formation of methyl esters[13, 14]. The industrial processing of shrimp in Sonora, México generates a large amount of remnant (byproducts), due to the inedible portion that is rejected as a food source (only 65% of the shrimp is edible). The rejected portion can become a serious environmental problem[15, 16]. Different techniques have been employed for the processing of the remnant such as acid and enzymatic hydrolysis, treatment with an organic acid, sun drying, and fermentation. Through a process of lactic fermentation, it is possible to recover high quality components, specifically, chitin, pigments and protein hydrolyzates. These by-products can be used for human consumption[17] and in the pharmaceutical, cosmetic and agricultural industries. The purpose of this study was to determine the content of lipids and the profile of fatty acids in the three forms of hydrolyzed protein obtained by lactic fermentation from shrimp heads. The possible application of the remnants in the food industry as sauces, flavoring flour, or fortifying supplements, could maximize the nutritional value of food. This work is part of a nutritional study to evaluate the biochemical and biological quality of the protein hydrolysates produced by the fermentation of shrimp byproducts.

MATERIALS AND METHODS

(i) Chemicals

Methanol, chloroform and hydrochloridric acid were purchased from Productos Químicos Monterrey (Monterrey, Nuevo Leon, Mexico). The anhydrous sodium sulfate was obtained from Fluka (USA). Toluene and potassium carbonate were purchased from Sigma (St. Louis, Missouri, USA). Fatty acids standard FAME Mix C8-C22 and PUFA No. 3 were obtained from Supelco (Bellefonte, Pennsylvania, USA).

(ii) Sample preparation

The research samples consisted of dry powder hydrolyzates, concentrated paste, and liquid hydrolyzates. The production of the liquid protein hydrolyzates was for lactic fermentation[18]. In this study, shrimp (*Penaeus*

spp.) remnant samples (heads and cephalothoraxes) were used. Slightly thawed minced remnants were fermented at 30 °C for 36 h. This silage was then centrifuged (5 °C) at 1250 rpm for 15 minutes to obtain the chitin-rich fraction (sediment), the liquid protein hydrolyzates, and the lipid fraction. In the production of the dry powder, the liquid hydrolyzates, which are rich in protein, was dehydrated using a spray dryer SD-04 Lab Scale Spry Drier (LabPlant, Huddersfield, West Yorkshire, England). The liquid hydrolyzates were transferred to a conical flask and placed in an electric grill heated to a constant 80 °C by the method of Bueno-Solano et al.(2009)[18]

(iii) Lipid extraction

The lipid content was extracted according to Sánchez-Machado et al. (2010)[19] with some modifications. The samples of dry powder (800 mg), liquid hydrolyzates (1 g) and concentrated paste (800 mg) were each placed in a 10 ml tube and diluted with 5 ml chloroform: methanol (2:1), then mixed for 1 min, sonificated for 6 min, and centrifuged for 5 min. The mixture was decanted into a tube at a constant weight. Finally, the sample was placed in an electric oven at 60 °C to achieve total evaporation. After evaporation, the weight difference determined the lipid content.

(iv) Fatty acid compositions

The profiles, of the fatty acid in the samples, were determined by gas chromatography[19]. Specifically, 500 mg of dry powder, 500 mg of liquid hydrolysates and 500 mg of concentrate were placed in separate tubes where 2 ml of toluene and 3 ml of 5% methanolic HCl were added (prepared daily). The samples were mixed in a vortex, and heated in a water bath for 2 h at 70°C. After that, the samples were cooled to room temperature, and 3 ml of 6% K_2CO_3 and 2 ml of toluene were added. The mix was agitated in a vortex, and then centrifuged at 2400 rpm for 5 min. The organic phase was dried with anhydride Na_2SO_4 and filtered with a 0.45 µm membrane. A 1µl sample was injected into the column of the GC system. Figure 1 shows the results of the chromatography of a hydrolyzed protein sample obtained by lactic fermentation from shrimp heads.

(v) Gas Chromatographic conditions

The analyses were carried out in duplicate, according to the methodology proposed previously[20], using gas chromatography (Varian 3800, Melbourne, Victoria, Australia) equipped with a flame ionization detector (FID), using a capillary column (60 m x 0.25 mm, Varian). The study used helium as the carrier gas; the temperatures of the detector and injector were 220 °C and 235°C, respectively. The column was maintained at 120°C for 1 min, then raised to 170°C at 3°C/min and maintained for 1 min, then raised to 235°C at 6°C/min and finally maintained for 5 min. One micro liter of sample was injected onto the column, and the fatty acids were identified by the comparison of the retention time of the PUFA No 3 standards (Supelco, Bellefonte, Pennsylvania, USA).

Gas chromatographic results

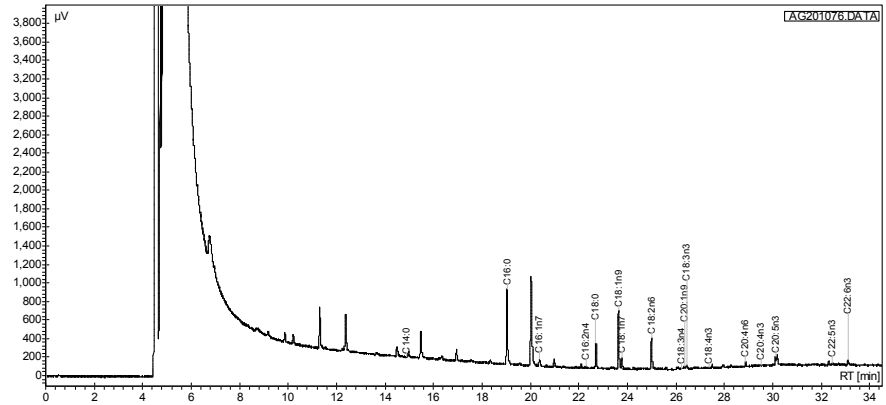

Figure 1
Chomatogram of a hydrolyzed protein as powder

RESULTS AND DISCUSSION

1. Lipid content in the three forms of hydrolyzates

The lipid content varied from sample to sample. A higher content of lipid was found in the protein powder with 36.78 %, and the lower content was in the liquid with 4.33%. The lipid content in the concentrate paste was in a range of 6.32 - 9.51% (wet weight). These high contents of lipids in all samples may be a result of the centrifugation of the silage. After the fermentation, the lipids were not completely removed from the liquid phase. In this study, the lipids in the liquid were separated manually, but with a mechanical separation system the lipid content could be lower. Also, due to the loss of moisture during the drying process and through evaporation, the lipid content was higher in the powder and the paste than in the liquid (Table 1). The content of lipids in the hydrolyzed portions are higher than in the contents of other research, for example, the hydrolyzate from shrimp shell [21] reported 1.1 to 2.2% of lipid in, but the technique for the recovery of the hydrolyzate protein was different. In shrimp head silage from fermentation[22], found a content of 3.61%. In shrimp shells treated enzymatically[23] reported 9.95% of lipid.

Table 1
Lipid content in the protein hydrolyzates (g per 100 g wet weight)[a]

Batch	Liquor	Concentrated paste	Powder
1	7.22 ± 0.71	6.32 ± 1.60	24.86 ± 0.22
2	7.65 ± 0.54	9.19 ± 1.02	24.78 ± 0.61
3	7.45 ± 0.98	6.90 ± 7.76	24.28 ± 0.10
4	7.75 ± 0.03	9.51 ± 0.81	36.78 ± 0.81
5	4.33 ± 0.65	8.65 ± 0.83	23.61 ± 0.93

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

This study was made to level laboratory, for this cause, the protein and lipid fractions were separated manually. Thus, the content the lipid is high in the hydrolyzates. The consequences of this problem are the diverse alterations that occur during the production and storage from hydrolyzates. One of these is the possible reduction of the nutritive value, and another is the production of volatile compounds that impart disagreeable odor and flavors in the final product. The amount of deterioration depends mostly on the type of lipids in the foods. In general terms, the lipid that can most easily be affected is found in seafood, followed by vegetable oils, and finally animal fats[1]. There is considerable evidence that therapeutic components, that can improve human health, are endogenous to food fats.

2. Profile of fatty acids

Table 2 shows the fatty acid profile of the three forms of hydrolyzate from the fermentation of shrimp heads. In this study, seventy fatty acids were identified with the total percentages of saturated, monounsaturated and polyunsaturated of 51.10%, 28.56% and 20.37% for the powder; 19.39%, 13.47% and 71.43%, for the liquid; and 41.25%, 23.80% and 36.74 % for the concentrated paste, respectively. In the three forms the hydrolyzates (powder protein, concentrated paste and liquid), the saturated fatty acid (SFAs) with the highest concentration was hexadecanoic acid (C16:0), while the highest concentration of a monounsaturated fatty acid (MUFA) was oleic acid (C18:1n9). Linoleic acid (C18:2n16) was the PUFA in highest concentration in the powdered protein and concentrated paste, and eicosapentanoeic acid (C20:5n3) was the highest in the liquid (Table 2). On the other hand, in the protein powder and the concentrated paste, the highest unsaturated fatty acids (HUFA) were eicosapentaenoic acid (EPA) and docosahexanoenoic acid (DHA), which represented 3% and 1.96% of the powder, and 5% and 2.91% of the concentrate, respectively. For both hydrolyzates, the powder and paste, the saturated fatty acids were higher than the monounsaturated fatty acids. In the liquid samples, the PUFAs were present in higher levels.

These values are similar to those reported in other research, for example, some reported that the oleic acid was most abundant in protein hydrolyzates from black scabbardfish by-products[24] and in salt-fermented shrimp paste[25]. Other reported that the saturated fatty acids, C16:0 and C18:0 were the most abundant fatty acids in the lipid extracted from black tiger shrimp and whit shrimp[4]. These values are similar to those obtained in this research where hexadecanoic acid was the fatty acid in higher levels. Seafood is known to contain a rich source of the n-3 FAs, especially EPA and DHA. DHA, in particular, plays a major role in several biochemical processes[7], and EPA has a beneficial effect on the cardiovascular system[26]. The three hydrolyzates have a good concentration of polyunsaturated fatty acids, which are considered anticholesterolemic[27]. The differences, among the samples, depend on the diet of the shrimp during growth, the kind of shrimp (heads), the fermentation batch, and the treatments incorporated in the production of the three classifications of hydrolyzates - liquid, paste and powder.

Table 2
Relative fatty acid contents of the three forms of hydrolyzates (% of total fatty acid content)[a]

Fatty acid[b]	Powder[a]	Liquor[a]	Concentrate paste[a]
C14:0	2.68	1.79	2.09
C16:0	37.82	12.55	31.44
C16:1n7	3.45	1.48	2.94
C16:2n4	0.33	0.17	0.24
C18:0	10.60	5.05	7.73
C18:1n9	19.73	9.70	14.75
C18:1n7	4.43	1.49	3.63
C18:2n6	11.75	8.54	10.47
C18:3n4	0.38	0.17	10.02
C20:1n9	0.95	0.80	1.47
C18:3n3	0.77	0.57	1.05
C18:4n3	ND	0.80	4.40
C20:4n6	1.75	1.59	1.95
C20:4n3	0.12	1.27	0.20
C20:5n3	3.00	50.74	5.00
C22:5n3	0.33	6.33	0.49
C22:6n3	1.96	1.26	2.91
∑ Saturate FAs	51.10	19.39	41.25
∑ monounsaturated FAs	28.56	13.47	23.80
∑ Polyunsaturated FAs	20.37	71.43	36.74

[a] Means values of n = 3, duplicate determinations; [b]FA = fatty acid

CONCLUSION

The hydrolyzed protein obtained, in addition to its high protein content, has a high percentage of lipids and a good concentration of fatty acid. In the powder and concentrated paste, the saturated fatty acids were present in higher concentrations. The fatty acid occurring in the higher level in the three forms was hexadecanoic acid. This research suggests that for both humans and animals can consider the hydrolyzate as a nutritious food supplement for his good content of n-3 fatty acid.

ACKNOWLEDGEMENT

This research was financed under project no. SON-2004-C03-016 with Mixed Funds of the Government of the State of Sonora and the National Council for Science and Technology (FOMIX-CONACYT).

REFERENCES

1. Badui S, Química de los Alimentos. 4th Edn, Ed. PEARSON, (2006).
2. Nagaraju-Patro M, Bankar, AS, Uttamrakesh S and Yadav AV, Oral lipid based formulation: a review. J Phar Biol Res, V1 (2):1-14, (2010)
3. Richards MP, Lipids Chemistry and Biochemistry. In: HUI, Y.H. Handbook of

food science, technology, and engineering: 8.1-8.21, (2006).
4. Montaño N, Gavino G and Gavino VC, Polyunsaturated fatty acid contents of some traditional fish and shrimp paste condiments of the Philippines. Food chem, 75 (4): 155-158, (2001).
5. Sriket P, Benjakul S, Visessanguan W and Kijroongrojana K, Comparative studies on chemical composition and thermal properties of black tiger shrimp (*Penaeus monodon*) and white shrimp (*Penaeus vannamei*) meats. Food chem, 103 (4): 1199-1207, (2007).
6. Ward OP and Singh A, Omega-3/6 fatty acids: alternative sources of production. Process Biochem, 40 (12): 3627-3652, (2005).
7. Økland HMW, Stoknes IS, Remme, JF, Kjerstad M and Synnes M, Proximate composition, fatty acid and lipid class composition of the muscle from deep-sea teleosts and elasmobranchs. Comp Biochem phys B, 140 (3): 437-443, (2005).
8. Chen D, Zhang M and Shrestha S, Compositional characteristic and nutritional quality of Chinese mitten crab (*Eriocheir sinensis*). Food Chem, 103 (4): 1343-1349, (2007).
9. Aued-Pimentel S, Mancio MM, Kumagai EE, Ruvieri V and Zenebon O, Comparison of gas chromatographic and gravimetric methods for quantization of total fat and fatty acids in foodstuffs. Quim Nova, 33 (1): 76-84, (2010).
10. González-Martín I, González-Pérez C, Alvarez-García N and González-Cabrera JM, On-line determination of fatty acid composition in intramuscular fat of Iberian pork loin by NIRs with a remote reflectance fibre optic probe. Meat Science, 69 (2): 243–248, (2005).
11. Sánchez-Camargo AP, Almeida-Meireles MA, Fontoura-Lopes BL and Cabral FA, Proximate composition and extraction of carotenoids and lipids from Brazilian red spotted shrimp waste (*Farfantepenaeus paulensis*). J Food Eng, 102 (1): 87–93, (2011).
12. Frega N, Bocci F and Lercker G, High-resolution gas-chromatographic determination of diacylglycerols in common vegetable oils. J Am oil Chem Soc, 70 (2): 175-177, (1993).
13. Meier S, Mjos SA, Joensen H and Grahl-Nielsen O, Validation of a one-step extraction/methylation method for determination of fatty acids and cholesterol in marine tissues. J Chromatogr A, 1104 (1-2): 291-298, (2006).
14. Pinto JSS and Lancas FM, Hidrólise do óleo de *Azadirachta indica* em água subcrítica e determinação da composição dos triglicerídeos e ácidos graxos por cromatografia gasosa de alta resolução a alta temperatura e cromatografia gasosa de alta resolução acoplada à espectrometria de massas. Quim Nova, 33 (2): 394-397, (2010).
15. López-Cervantes J, Sánchez-Machado DI and Rosas-Rodríguez JA, Analysis of free amino acids in fermented shrimp waste by high-performance liquid chromatography. J Chromatogr A, 1105 (1-2): 106-110, (2006).
16. Guilherme RF, Cavalheiro JMO and Simao De Souza PA, Chemical characterization and profile of the amino acids of the flour of shrimp head, Ciência e Agrotecnologia, 31 (3): 793-797, (2007).
17. Je J, Qian Z, Byun H and Kim S, Purification and characterization of an antioxidant peptide obtained from tuna backbone protein by enzymatic hydrolysis. Process biochem, 42 (5): 840-846 , (2007).
18. Bueno-Solano C, López-Cervantes J, Campas-Baypoli ON, Lauterio-García R, Adan-Bante NP and Sánchez-Machado DI, Chemical and biological characteristics of protein hydrolysates from fermented shrimp by-products. Food chem, 112: 671-675, (2009).
19. Sánchez-Machado DI, Núñez-Gastélum JA, Reyes-Moreno C, Ramírez-Wong B and López-Cervantes J, Nutritional quality of edible parts of *Moringa oleifera*. Food analytical methods, 3: 175-180, (2010).
20. Núñez-Gastélum JA, Sánchez-Machado DI, López-Cervantes J, Paseiro-Losada P, Sendón R, Sanches-Silva AT, Costa HS, Aurrekoetxea GP, Angulo I and Soto-Valdez H, Physical and chemical properties of pigmented oil obtained from shrimp heads. International journal of fats and oils, 62 (3): 321-327, (2011).

21. Shahidi F and Synowiecki J, Isolation and characterisation of nutrients and value-added products from Snow Crab (*Chinoecetes opilio*) and Shrimp (*Pandalus borealis*) processing discards. J Agr Food Chem, 39 (8): 1527-1532, (1991).
22. Nwanna LC, Risk Management in Aquaculture by Controlled Feeding Regimen. Pakistan Journal of Nutrition, 2 (6): 324-328, (2003).
23. Synowiecki J and Al-Khateeb N, The recovery of protein hydrolysate during enzymatic isolation of chitin from shrimp Crangon crangon processing discards-commercial uses and potential applications. Food chem, 68 (2): 147-152, (2000).
24. Batista I, Ramos C, Coutinho J, Bandarra NM and Nunes ML, Characterization of protein hydrolysates and lipids obtained from black scabbardfish (*Aphanopus carbo*) by-products and antioxidative activity of the hydrolysates produced. Process biochem, 45(1): 18-24, (2010).
25. Peralta EM, Hatate H, Kawabe D, Kuwahara R, Wakamatsu S, Yuki T and Murata H, Improving antioxidant activity and nutritional components of Philippine salt-fermented shrimp paste through prolonged fermentation. Food chem, 111: 72-77, (2008).
26. Anitha U and Karuppasamy R, Sesame meal administration attenuate the high-fat diet induced lipid abnormalities and improve insulin sensitivity in wistar rats. J Phar Biol Res, 1 (3): 273-280, (2011).
27. Bragagnolo N and Rodriguez-Amaya DB, Total Lipid, Cholesterol, and Fatty Acids of Farmed Freshwater Prawn (*Macrobrachium rosenbergii*) and Wild Marine Shrimp (*Penaeus brasiliensis*, *Penaeus schimitti, Xiphopenaeus kroyeri*), J Food compos anal, 14 (4): 359-369, (2001).