Microwave extraction of proteins from *Litopenaeus vannamei* molt shell using only water as a solvent

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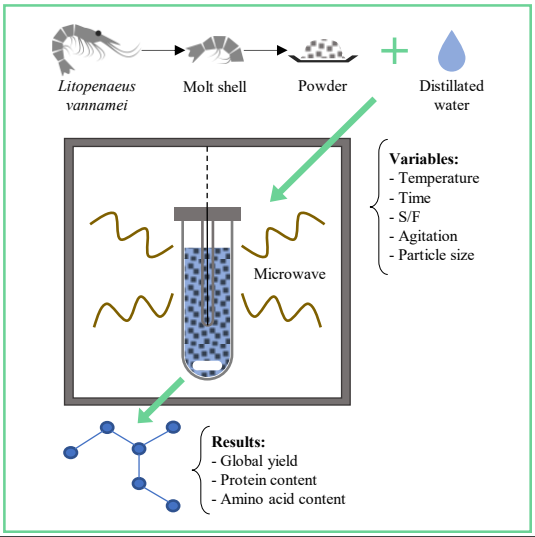

This study aimed to apply microwave technique for the extraction of proteins from shrimp molt shell using water as the only solvent. The variables temperature, isothermal time, particle size, solid-liquid ratio (S/F) and stirring rate were studied to understand if they are significant variables. By varying the process parameters, it was possible to obtain an extraction yield of 16-31g/100g dry molt shell, extracted proteins of 3-7 g/100g of dry molt shell, and extracted amino acids of 0.5-3 g/100 g dry molt shell. In addition, isothermal time was found to be the variable with the highest influence on the extracted protein content. The obtained results indicated the variables temperature, isothermal time, and S/F as significant variables for protein extraction, within the selected range.

Introduction

Crustacean production in 2022 represented 10% of world fisheries and aquaculture [1]. The consumption and production of shrimps generates as waste the cephalothorax and its exoskeleton (shell) [2]. The exoskeleton represents approximately 47% of the animal [2], and is a potential problem during the aquaculture process, since during the growth phases the molting process occurs with the exchange of the shell, releasing the old shell to the growth medium. These molt shells are not commonly valued, compared to the shell of the adult animal [3]. Currently the shrimp shell is used to obtain chitin [4]. However, the shell also contains other compounds of commercial interest, such as proteins (20-40%) [4]. During the conventional process, the proteins are separated from the shell using alkaline solutions (commonly sodium hydroxide solutions), which is not a sustainable process, and the protein obtained in this way cannot be reused [5]. To make the chitin extraction process more sustainable, some authors have studied the application of microwave technology, making the process more effective [6,7]. However, many of these processes use inorganic bases as extraction solvent.

Objective

In this context, this project aimed to study the use of microwave technology with only water as extraction solvent to obtain shrimp molt shell proteins.

Methods

For the extraction processes, the shrimp molt shell (supplied by the company Noray Seafood, Valladolid, Spain) was previously washed, dried in an oven at 105 °C for 24 h and grounded with a knife mill (SM 100, Retsch, Haan, Germany). The shell powder was initially characterized in terms of ash, protein, amino acids, fatty acids, and chitin content (Table 1).

A microwave oven with magnetic stirring and sensors of pressure and temperature (Monowave 300, Anton-Paar, Madrid, Spain) was used for all the experiments in closed system. Particle size distribution was evaluated by DLS (Mastersizer 2000, Malvern Panalytical, Malvern, UK). Stirring rate (N: 600 and 1000 rpm), temperature (T: 175 and 225 °C), isothermal time (t: 0, 10 and 20 min), solid-liquid ratio (S/F: 10 and 20 mL/g) and particle size (d_{90}: 483 and 125 µm) were studied as variables. The products were analyzed by the pH variation, extraction yield (X_0), proteins by BCA (bicinchoninic acid assay), and amino acids (AA) by ninhydrin method, all of them expressed as g per 100 g of dry molt shell. The process was carried out in duplicate. The results were statistically evaluated in the software statgraphics 19® 19.4.01 version (Statgraphics Technologies, Inc., VA, US) by one-way analysis of variance (ANOVA) with a statistical significance level of 5% by Tukey's test.

Table 1. Chemical composition of the shrimp molt shell

Compound	(g/100 g dry molt shell)
Ash	53.7 ± 0.5
Protein	10.6 ± 0.4
Amino acid	1.37 ± 0.12
Fatty acid	3.8 ± 0.9
Chitin	17.8 ± 0.7

Results

Adding the raw material to the water (without applying any process) increased the pH of the water, making it alkaline (9.53 ± 0.08), probably related to the release of minerals from the shell ($CaCO_3$). After the extraction processes the pH decreased to (8.8 ± 0.2), which may be related to the release of proteins and other compounds (chitin and derivatives). This analysis indicates the possibility of using water instead of inorganic bases for this raw material, since the minerals contained in the shell can basify the medium.

For the effect of stirring rate the results indicate that increasing the rotation did not show any significant effect in terms of extracted proteins (6.1 ± 0.3 g per 100 g of dry molt shell at 600 rpm and 6.2 ± 0.2 g per 100 g of dry molt shell at 1000 rpm) and a lower stirring rate showed higher co-extraction (31 ± 4 g per 100 g of dry molt shell at 600 rpm and 28.2 ± 0.7 g

per 100 g of dry molt shell at 1000 rpm) and dispersion of the data. Therefore, a stirring rate of 1000 rpm was fixed to study the rest of variables. For the evaluation of the temperature effect, the results indicate that in terms of extracted protein, the temperature had significant influence in this studied range (Table 2). Total proteins, considered as the sum of BCA analysis and AA increased from 7.4 ± 0.55 to 9.6 ± 0.4 g /100 g of dry molt shell. Increasing temperature promotes a significant increase in the content of free amino acids, which suggests a breakdown of the protein chain under such conditions (combination of temperature and time). In terms of global yield, results have shown that a higher co-extraction of other compounds may have occurred for a temperature of 225 °C.

Table 2. Effect of Temperature. (N = 1000 rpm, t = 10 min, S/F = 20 mL/g, d_{90} = 483 µm)

T (ºC)	X_0	Extracted Proteins	AA
	(g/100 g of dry molt shell)		
175	22.8 ± 0.9[a]	6.6 ± 0.5[a]	0.80 ± 0.05[a]
225	28.2 ± 0.7[b]	6.2 ± 0.2[a]	3.4 ± 0.2[b]

[a,b] Different lowercase letters in the same column indicate significant difference ($p < 0.05$).

Isothermal time was counted only after reaching the studied temperature, being the heating periods shorter than 90 s in all the experiments, where the equipment adapted the microwave power based on the set temperature to have rapid heating. As is shown in Table 3, the time of 10 and 20 minutes did not show significant differences for the X_0. However, in terms of extracted protein and amino acid, the 10 min time performed better than the 20 min time, and it is possible to see a tendency for increased protein breakdown with a longer isothermal time. Based on these results it was possible to verify that the time of 20 min seems to be a long time in this temperature.

Table 3. Effect of isothermal time. (T = 175 ºC, N = 1000 rpm, S/F = 20 mL/g, d_{90} = 483 µm)

t (min)	X_0	Extracted Proteins	AA
	(g/100 g of dry molt shell)		
0	16.8 ± 0.4[a]	3.5 ± 0.2[a]	0.54 ± 0.02[a]
10	22.8 ± 0.9[b]	6.6 ± 0.5[b]	0.80 ± 0.05[b]
20	23.9 ± 0.6[b]	5.1 ± 0.5[c]	1.06 ± 0.11[c]

[a,b,c] Different lowercase letters in the same column indicate significant difference ($p < 0.05$).

The effect of particle size has been studied modifying milling conditions to obtain two particle sizes: d_{90} = 483 and 125 µm, and the results are included in Table 4. Based on the results (Table 4) it was verified that the reduction in the particle size has no effect on the improvement of the extracted protein content, under the studied conditions, however the decrease in particle size has led to higher co-extraction of other compounds. Considering this co-extraction, the higher-energy demand of the milling pretreatment to achieve smaller particle size, and the difficulties derived to handle this smallest particle size in subsequent downstream steps, the particle size of 483 µm was selected for the next studies.

Table 4. Effect of particle size. (T = 175ºC, N = 1000 rpm, t = 10 min S/F = 20 mL/g)

d_{90} (µm)	X_0	Extracted Proteins	AA
	(g/100 g of dry molt shell)		
483	22.8 ± 0.9[a]	6.6 ± 0.5[a]	0.80 ± 0.05[a]
125	28.4 ± 1.2[b]	5.8 ± 0.3[b]	0.92 ± 0.05[b]

[a,b] Different lowercase letters in the same column indicate significant difference ($p < 0.05$).

For the S/F analysis, the S/F was varied at 10, 20 and 40 mL/g, and results are shown in Table 5. The results indicate that increasing the S/F from 20 to 40 led to a higher co-extraction.

Table 5. Effect of S/F. (T = 175ºC, N = 1000 rpm, t = 0, d_{90} = 483 µm)

S/F (mL/g)	X_0	Extracted Proteins	AA
	(g/100 g of dry molt shell)		
10	16.4 ± 0.5[a]	2.5 ± 0.2[a]	0.58 ± 0.06[a]
20	16.8 ± 0.4[a]	3.5 ± 0.2[b]	0.54 ± 0.02[a]
40	18.65 ± 0.04[b]	4.2 ± 0.4[c]	0.58 ± 0.03[a]

[a,b] Different lowercase letters in the same column indicate significant difference ($p < 0.05$).

The results obtained in these experiments demonstrated that temperature, isothermal time, and S/F are variables that need to be studied in an experiment design to optimize operational condition for microwave assisted extraction of proteins using only water.

Conclusions

This study showed that the use of microwave technology with water as the only solvent is an effective method to extract proteins from shrimp molt shells with the possibility of using these proteins in industrial sectors, which is a differential of this technique compared to the conventional alkaline extraction method. Besides that, this technique presents shorter process times and does not generate harmful waste compared to the conventional process. Following these results, an experimental design is being carried out and experimental results will be shown during the congress. In addition, a study will also be conducted to identify the co-extracted compounds, and the molecular weight of the extracted proteins.

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