SEASONALITY STUDY OF *Penaeus vannamei* SHRIMP SHELLS FROM AQUACULTURE

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**ABSTRACT**

Chitin and chitosan have nowadays numerous industrial applications in various areas of engineering, but its industrial production and consolidation as a raw material is conditioned by fluctuations in both shrimp seasonality not allowing proper process optimization. One way to bridge this gap would be through the use of shrimp from aquaculture. Thus the present study aims to determine whether the shell from shrimp aquaculture has its characteristics changed over a calendar year. Shells from the processing of *Penaeus Vannamei* shrimp grown in farm was collected for over an year and characterized for its composition, having determined that the shell has an average of 10% residual moisture, 35% minerals, 35% proteins and 20% of chitin. Additionally, the chitin extracted was characterized for its extrinsic viscosity and degree of acetylation, which were averaged 200cps and 93% respectively. From the results, it was found that there isn’t a significant variation in the composition of the shrimp shells nor the chitin characteristics and is therefore deemed that this source of chitin is suitable to drive chitin and chitosan production industry.

**Keywords:** waste treatment, seasonality, chitin extraction, chitosan, northeast Brazil

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**ESTUDO DA SAZONALIDADE DA CASCA DO CAMARÃO *Penaeus vannamei* NA AQUICULTURA**

**RESUMO**

A quitina e a quitosana têm hoje numerosas aplicações industriais em diversas áreas da engenharia, mas sua produção industrial e consolidação como matéria-prima é condicionada por flutuações na sazonalidade do camarão, não permitindo a otimização adequada do processo. Uma forma de solucionar esta lacuna seria através do uso de camarão da aquicultura. Assim, o objetivo deste estudo foi determinar se a casca de camarão produzido em viveiros tem suas características alteradas ao longo de um ano civil. As cascas do processamento do camarão *Penaeus vannamei* cultivadas em fazendas foram coletadas por mais de um ano e sua composição caracterizada, tendo-se determinado que a casca tem uma média de 10% de umidade residual, 35% de minerais, 35% de proteínas e 20% de quitina. Adicionalmente, a quitina extraída foi caracterizada pela sua viscosidade extrínseca e grau de acetilação, que foram calculados em média de 200 cps e 93%, respectivamente. A partir dos resultados, verificou-se que não há uma variação significativa na composição das cascas do camarão nem nas características da quitina e, portanto, esta fonte é considerada adequada para implantar uma indústria de produção de quitina e quitosana.

**Palavras-chave:** tratamento de resíduos, sazonalidade, extração de chitina, quitosana, nordeste do Brasil

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INTRODUCTION

Shells from crustaceans are an underutilized abundant solid waste from food industry, with a negative environmental impact that stems largely from shrimp processing industry using aquaculture as the main provider (Green & Mattick, 1977; Primavera, 2006). Globally, 3,668,681 tons of shrimp from the species Penaeus vannamei (P. vannamei), were produced by aquaculture in 2014. In northeast of Brazil the same specie predominates in 95% of aquaculture farms and this region accounts for about 92% of all production totaling about 80 thousand tons in 2010 (FAO, 2014; Antonino, 2007). The species P. vannamei or white shrimp is also one of the three species most cultivated in the world, along with the Penaeus monodon and Penaeus chinensis (FAO, 2014; Cuhu, et al. 2012).

Chitin is the second most abundant polysaccharide in nature, followed by cellulose, and its main source of commercial extraction are the shells of crustaceans (Gaf, 1992; Muzzarelli, 1977; Rinaudo, 2006). This biopolymer is the main raw material for the production of chitosan and derivatives thereof, such as glucosamine which is widely accepted in the dietetic industry (Chen, 2010; Kumar, 2000). Additionally, there are several products made from chitin and chitosan, with applications ranging from the water treatment to the biomedical industry (Bhatnagar & Sillanpää, 2009; Yang, 2016; Dash, et al., 2011). Extraction of chitin and chitosan production implies the treatment of shrimp shells which in its 90% of total dry weight have three main components in their constitution: chitin, protein and minerals (Raabe, et al., 2005).

Even though there are alternatives, the commercial extraction of chitin is mainly done by chemical treatments (Younes & Rinaudo, 2015). The first step is a demineralization, using a strong acid, typically HCl for solubilization of minerals and then a second step comprising the deproteinization, that uses a strong base, typically NaOH to solubilize proteins. (Percot, et al., 2003; Cavalcante, et al., 2013) Chitosan is produced by alkaline hydrolysis during the deacetylation step of chitin, where a strong base, typically NaOH at high concentrations around 50 wt% reacts with chitin acetyl group forming the deacetylated form of chitin (Lamarque, et al., 2004). All these steps have an impact on the parameters that influence the behavior of the biopolymer in their applications, such as the deacetylation degree, molecular weight and more decisive the purity (Trung, et al., 2006; Aranaz, et al., 2009).

Although it is considered a raw material with such potential and wide application, high manufacturing costs associated with low productive capacities limit the growth of these biopolymers on the market. Additionally, applications on the food industry, the biomedical or cosmetic require products with high purity and uniform quality, which is not true for the chitin and chitosan, always existing fluctuations even from batch to batch (Youn, et al., 2009; Cho, et al., 1998). The reasons for these fluctuations and lack of uniformity may be associated with the difficulty of establishing an optimized and economic process of a shell with the same characteristics and availability for at least one calendar year. Experts consider this to be the main reason for chitosan not thrive in biopolymers market, unlike others such as cellulose or starch (Khor, 2002). The present work aims to study the shells from processed shrimps farmed at an aquaculture industry and determine if whether it maintains the same composition over a calendar year.

MATERIALS AND METHODS

Preparation and Characterization of Peel

Shrimp shells (P. Vannamei) obtained at each quarter of the year were collected frozen at Aquamaris, João Pessoa, Brazil. Shells were first thawed, the heads discarded, and the body parts were then dried using a tray oven at 40 ° C until constant weight. After complete drying, the shells were ground using a blade mill with a mesh of 500μm.

Dry weight and ash content

For determining the dry weight, shell samples of approx. 5g were added to a ceramic crucible and dried at 105 °C for 24h, whereas for determining the ash content the same samples were further burned using a chamber furnace at 530 ° C for 20h. After weighing, the ash samples were analyzed by Scanning Electronic Microscopy (SEM) / Energy-dispersive X-ray spectroscopy using a X Phenom PRO equipment for determining and quantifying the mineral composition.
Extraction of Chitin

Chitin was extracted from 10 g samples of dried ground shrimp shell, obtained from the four quarters of the year, through a process of demineralization performed with aqueous 1.0 M hydrochloric acid (HCl, Sigma-Aldrich, Pa) for 24 hours at room temperature, and with a ratio solid / liquid of 1/40 (1 g of shells per 40 mL of aqueous 1.0 M HCl). After filtration, the demineralized shells were washed with distilled water until neutral pH was achieved, and then dried using a tray oven at 40 °C until constant weight. The demineralized shell was then deproteinized with aqueous 1.0 M sodium hydroxide (NaOH, Sigma-Aldrich, Pa) for 24 hours at 60 °C and with the same solid / liquid ratio. At the end the product was washed with distilled water until neutral pH and then treated with ethanol to remove the remaining pigments. The chitin thus obtained was dried at 40°C until constant weight was achieved.

Table 1 - Dry weight and ash present on P. Vannamei shells

<table>
<thead>
<tr>
<th>Month</th>
<th>Initial Weight (g)</th>
<th>Dry Weight (g)</th>
<th>Residual water (%)</th>
<th>Total Ash (g)</th>
<th>Total Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>5,007</td>
<td>4.5441</td>
<td>9.24</td>
<td>0.8279</td>
<td>18.24</td>
</tr>
<tr>
<td>April</td>
<td>5,017</td>
<td>4.5487</td>
<td>9.33</td>
<td>0.8320</td>
<td>18.33</td>
</tr>
<tr>
<td>July</td>
<td>5,011</td>
<td>4.5392</td>
<td>9.41</td>
<td>0.8408</td>
<td>18.48</td>
</tr>
<tr>
<td>October</td>
<td>5,009</td>
<td>4.5384</td>
<td>9.39</td>
<td>0.8363</td>
<td>18.40</td>
</tr>
<tr>
<td>Average</td>
<td>-</td>
<td>-</td>
<td>9.34</td>
<td>0.8342</td>
<td>18.33</td>
</tr>
</tbody>
</table>

Characterization of chitin

Chitin samples were characterized for its extrinsic viscosity by first dissolving it in a solution comprising DMAC + LiCl 2 5%wt at a concentration of 1%wt. The solution without suspended particles was characterized using a Brookfield DV II + PRO, and the viscosity values were recorded at 60 rpm. Chitin samples were also characterized by FTIR-ATR (Perkin Elmer Nicolet) at wavelengths of 650-4000 cm\(^{-1}\) with a resolution of 4 cm\(^{-1}\) and 64 scans. To determine the degree of acetylation, equation 1 was used as described by Brugnerotto (Brugnerotto, et al., 2001).

\[
\frac{A_{350}}{A_{420}} = 0.3822 + 0.03133DA \quad \text{Equation 1}
\]

RESULTS AND DISCUSSION

P. Vannamei shell characterization

Results obtained for the characterization of shrimp shells are summarized in Table 1. By analyzing values for the percentage of residual water, the average value was 9.34 ± 0.07% without significant differences throughout the year. After removing residual water the samples were burned for ash content determination. Average values are 18.36 ± 0.1% without evidence of significant variations during the year. Charoenvuttitham and colleagues report values of 23% for ash content of shells from the species *Penaeus Monodon* and Sagheer report values of 29% for *Penaeus Semisulcatus*. (Charoenvuttitham, et al., 2006; Al Sagheer, et al., 2009).

EDX was used to determine the composition of the ash and the elements detected by such method were Ca, Na, P, Mg, K. Calcium(Ca) was the most abundant element among the minerals present in ash as described in Table 2. Calcium which is in the form of calcium carbonate (CaCO\(_3\)) has an average value of 15.85% of the ash, followed by 1% of Phosphorus (P) and 0.69% of magnesium. It was also found Potassium and Sodium. Most researchers found similar elements and in all studies Calcium is the most abundant element. (Percot, et al., 2003; Rodde, et al., 2008; Shahidi & Synowiecki, 1992). Additionally it was found that the variation over the course of each of the elements in the shrimp shells is considered small.
Table 2 - Chemical composition of each mineral on *P. Vannamei* shells

<table>
<thead>
<tr>
<th>Month</th>
<th>Ca (%)</th>
<th>Na (%)</th>
<th>P (%)</th>
<th>Mg (%)</th>
<th>K (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>15,79</td>
<td>0,51</td>
<td>1,00</td>
<td>0,69</td>
<td>0,24</td>
<td>18,24</td>
</tr>
<tr>
<td>April</td>
<td>15,80</td>
<td>0,53</td>
<td>0,97</td>
<td>0,75</td>
<td>0,27</td>
<td>18,33</td>
</tr>
<tr>
<td>July</td>
<td>15,91</td>
<td>0,50</td>
<td>1,03</td>
<td>0,78</td>
<td>0,26</td>
<td>18,48</td>
</tr>
<tr>
<td>October</td>
<td>15,88</td>
<td>0,52</td>
<td>1,05</td>
<td>0,72</td>
<td>0,24</td>
<td>18,40</td>
</tr>
<tr>
<td>Annual Average (%)</td>
<td>15,85</td>
<td>0,51</td>
<td>1,01</td>
<td>0,73</td>
<td>0,25</td>
<td>18,36</td>
</tr>
</tbody>
</table>

**Chitin extraction**

Chitin was extracted to determine if whether its characteristics vary throughout the year. For better comparison the same extraction conditions were used in all samples with the purpose of complete removal of mineral and protein and ensure comparability between the yields obtained in each extraction. After chitin extraction new ash content test was performed but no amount of residue was found, and it is considered that the removal of mineral was complete. In addition, we analyzed the absorption band at 1540 cm\(^{-1}\) and no band was detected so according to Blackwell (Blackwell, 1988) the absence of such absorption band denotes absence of proteins in the chitin structure. Thus, with the yields obtained it is possible to determine the percentage of proteins present in the shell. Figure 1 depicts the fractions extracted in the demineralization step and deproteinization step along with the final portion of chitin in the shells. Shells from July have a higher amount of mineral content, 37.9 ± 1.2% and shells from January have an higher amount of proteins, 40.4 ± 2.5%. Similar extraction data were obtained by Charoenvuttitham for *Peneaus Monodon* (Charoenvuttitham, et al., 2006). Throughout the year, the quantity of minerals is always smaller than the amount of protein present in the shells and with minor variations. The discrepancy between the values obtained in demineralization and ash content is related to the carbonated form of minerals, which by reacting with hydrochloric acid release carbon dioxide gas forming in the case of Calcium, Calcium Chloride (Tolaimate, et al., 2003). The same discrepancy was observed in Xu and co-workers work for *P. Monodon* (Xu, et al., 2008). During demineralization, a large portion of pigments and lipids are also removed by oxidation, although according to Rodde and co-workers, this is a negligible fraction ranging from 14 to 39 mg / kg and 0.3% to 0.5% respectively [5,27]. Finally, shells from October have the largest amount of chitin with a value of 28.7 ± 1.5% but no evidence of significant variations were found during the year.
Chitin characterization

One of the biggest problems while preparing pure chitin is the degradation suffered during the extraction procedure (Gaf, 1992). The molecular weight is affected by the extraction conditions due to breakage of the glycoside linkage and subsequent depolymerization caused by contact with hydrochloric acid and hydroxide sodium (Younes & Rinaudo, 2015). Since extrinsic viscosity is dependent of molecular weight along with chitin concentration, using the same extraction conditions was also important for comparison of this parameter (Baxter, et al., 2005). Similarly to extrinsic viscosity, the degree of acetylation decreases by the action of sodium hydroxide due to the reaction with the acetyl group at the amine group in C-2.

In Figure 2 are depicted the values of the extrinsic viscosity and degree of acetylation of chitin obtained throughout the year. The average value of extrinsic viscosity throughout the year was 190.8 ± 2.5 cps but no significant variation from quarter to quarter was identified. Higher results were obtained by Bajaj and colleagues for the shrimp shell of the species Crangon crangon (Bajaj, et al., 2011). In that work the extrinsic viscosity values are obtained for chitin with smaller times for demineralization and deproteinization and so the result is expected to be lower. Theoretical values for the degree of acetylation are 100% in the untreated native chitin in shrimp shell, but its value is reduced by reaction with sodium hydroxide (Kurita, 2006). However, the values remain high, with an average of 88.2% and reduced variation throughout the year. Most researches find values for the degree of acetylation within a typical range of 100 to 85% (Gaf, 1992; Percot, et al., 2003). The extended time for deproteinization might be the cause for the low value of %DA.

CONCLUSION

On the present work it was successfully determined the composition of shrimp shell from shrimp species P. Vannamei produced in aquaculture over a year. For the same period of time, chitin from the same shells was also extracted and characterized. In both cases there were no seasonal variations which means that shells from aquaculture shrimps could potentially help solving the issues hindering chitin and chitosan production and usage at larger scales.

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