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Calcium Salts from the Demineralization of Crab (*Scylla serrata*) Wastes

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ABSTRACT

Seafood wastes raise environmental concerns and many studies focus on recycling those wastes into chitosan. However, the environmental impact of that process is underestimated. Indeed, a significant amount of toxic wastes is released, potentially harmful to humans and the environment. This study therefore focuses on reducing of that adverse impact by recycling the waste produced during the first step of the production of chitosan: demineralization. By varying the concentration of the acid while keeping stoichiometric ratios, it was shown that the concentration plays a vital role in the efficiency of the demineralization and the purity of the salts. This effect of the concentration on such reaction is still poorly investigated and this study thus offers a deeper knowledge to the chitosan research. Moreover, it was concluded that calcium salts from the transformation of crab wastes into chitosan can be collected and reused. Nevertheless, studies about recycling waste from the other steps of the chitosan production are needed to reduce this process's environmental impact even more.

INTRODUCTION

The waste generated by the seafood industry is about 10^6 tons per year (Schmitz *et al.*, 2019). Crustaceans contain about 40% meat, the remaining 60% inedible part raises questions within the industry about the scale of crustacean waste accumulation (Amiri *et al.*, 2022). Not only do those wastes cause a significant part of the contamination of the environment, but they also release unpleasant odors, attracting and stimulating the proliferation of insects (Wang and Nguyen, 2019). Since they are characterized by their high concentrations of nitrogen, phosphorus, organic carbon, suspended solids and oxygen, effluents from the fishing industry, if discharged into the environment without prior treatment, cause physical and chemical changes in aquatic environments, which can lead to the mortality of aquatic animals and impact local microfauna and microflora (Wang and Nguyen, 2019). Most of this waste is destined for composting or transformed into low added value products such as animal feed and fertilizer (Schmitz *et al.*, 2019).

Chitin is the second most abundant natural polymer after the cellulose, and chitosan is its most important product or derivative. Chitosan has several properties: nontoxic, biodegradable, biocompatible and antimicrobial. Therefore, it makes its way through scientific research in many different fields (Kumar, 2019; Ahmed and Ikram, 2015). In this context, approximately 2000 tons of chitosan are produced annually, the main source of extraction being from shrimp and crab shell residues (Muñoz *et al.*, 2018). Contrarily most petroleum-based polymers, which are neither renewable nor biodegradable, chitosan allows the implementation of a circular materials economy by developing environmentally sustainable materials (Bai *et al.*, 2022).

However, to isolate chitin from shrimp waste chemically, an acid is used for demineralization and NaOH for deproteinization. That process releases large amounts of toxic waste that without further treatment would pollute the environment (Oh *et al.*, 2007). The demineralization step consists of removing minerals, mainly the calcium. In a previous study (Andriamanalina *et al.*, 2023), the salts resulting from the demineralization of crab wastes were successfully used in self-healing concrete.

The principal objective of this present work is to produce usable salts from crab waste and thus, reducing the environmental impact of the production of chitosan. This paper studies the optimization of the conversion of calcium inside the crab wastes into salt and the evaluation of the purities of the collected salts.

MATERIALS AND METHOD

Crab wastes samples

Crab (*Scylla serrata*) wastes were provided by the ANTARTICA society (Antananarivo, Madagascar). Their compositions are presented in Tab. 1 and Tab. 2. The crab wastes were dried, grinded and sieved to obtain 0.2-1mm grain size. Figure .1 shows the process to obtain the chitosan. The calcium salts from the demineralization will be further collected.

Preparation of calcium salts

To prepare the salts control, calcium carbonate (ROTH, Assay≥98.5%)and lactic acid (RESEARCH-LAB, Assay 88,0-92,2%, d=1,20-1,21) were mixed in stoichiometric proportions; the same procedure was applied for acetic acid (Acetic acid: MERCK, Assay 99,8%, d=1,05). The crab wastes were treated with the two acids in the same ways, replacing the weight of $CaCO_3$ with the equivalent weight of $CaCO_3$ present in crab wastes. The solutions, reduced to 100 ml, were stirred at 100 rpm for 24 h at 30° C, then centrifuged at 3000 rpm for 10 min. Various acid concentrations (0.25 M; 0.5 M; 1 M) were applied while keeping the stoichiometric proportions.

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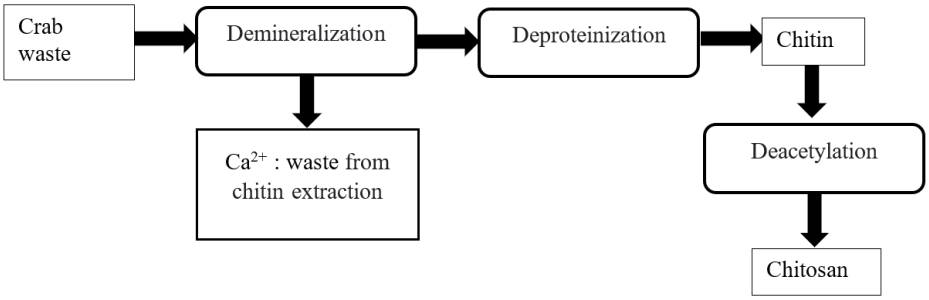

Figure 1: Chitosan production process

Table 1: Composition of crab waste (Rakotondravelo, 2019)

Component	Content
Moisture	7,91%
Nitrogen	4,26%
Proteins	26,64%
Lipids	1,28%
Ash	53,25%

Table 2: Mineral composition of crab waste (Rakotondravelo, 2019)

Mineral element	Content
Calcium (Ca)	16,70%
Magnesium (Mg)	1,29%
Phosphorus (P)	1,04%
Potassium (K)	0,41%
Iron (Fe)	0,08%

Conversion rate

In order to determine the conversion rates, the concentrations of calcium ions in solution were evaluated by atomic absorption spectrometry (VARIAN SpectrAA.20) with a wavelength of 424 nm. The solutions were diluted 500 times or 2500 times, and a buffer solution of lanthanum oxide (58.6 g/l of lanthanum oxide + 25% HCl 12 M) was added at a rate of 10% of the volume.

$$\tau = \frac{c \times f \times M_{CaCO3} \times V}{m_{CaCO3} \times M_{Ca}}$$

where

τ: Conversion rate; c: Measured calcium concentration; f: Dilution factor; M_{CaCO3}: Molar mass of calcium carbonate; V: Volume of the solution; m_{CaCO3}: Mass of calcium carbonate before reaction or present in the crab initially; $M_{Ca:}$ Molar mass of calcium

Purity of the salts

The solutions were dried at 60°C, the salts were collected in the oven and immediately hermetically packaged. Indeed, the dried salts from crab waste were highly hygroscopic and became pasty and sticky in contact with air. Their purities were evaluated by drying the solution resulting from the dissolution of crude $CaCO_3$ or from the demineralization of crab wastes, according to:

$$p = \frac{c \times V_{Sol} \times M_{Salt}}{m_{exp} \times M_{Ca}}$$

where

p: Purity of the salt obtained (calcium lactate or calcium acetate); c: Measured calcium concentration $V_{Sol:}$ Volume of the solution to be dried; $M_{Salt:}$ Molar mass of the salt obtained; $m_{exp:}$ Measured salt mass

Statistical analysis

A 3-way analysis of variance (ANOVA) followed by a Bonferroni post-hoc test was used to identify significant differences between the conversion rates

- from $CaCO_3$ and crab,
- by lactic acid and by acetic acid,
- and from different concentrations

The same process was used for the purities of the salts. Data were statistically analyzed using the XLSTAT software (Addinsoft Corporation) (Addinsoft, 2014).

RESULTS AND DISCUSSIONS

Calcium conversion rate

The conversion rate reflects the yield of the obtained salts. In order to optimize that, the acid concentration was varied from 0.25 M; 0.50M; and 1.00 M and the conversion rates resulting from the actions of lactic acid and acetic acid on $CaCO_3$ and crab wastes were evaluated. Figure. 2 show the behavior of the experimentally measured conversion rates according to the substrate ($CaCO_3$ or crab waste), according to the reagent (lactic acid or acetic acid) and according to the concentration (1.00 M, 0.50 M, 0.025 M). The conversion rates of raw $CaCO_3$ are significantly $(p<0.0001)^{\alpha=0,05}$ high compared to those of $CaCO_3$ in crabs.

In the crab, parallel reactions can take place with the other minerals. In addition, the complex nanofiber-like structure interacts with reaction rates and non-reactive materials such as proteins and chitin prevent fluid from moving freely to the reaction zone (Fadli *et al.*, 2017).

The conversion rates by lactic acid are significantly $(p<0.0001)^{\alpha=0,05}$ high compared to those by acetic acid.

The high conversion by lactic acid is explained by the fact that the conversion rate of calcium depends on the concentration of H_3O^+ which depends on the strength of the acid. As the strength of lactic acid (pKa = 3.86) is higher than that of acetic acid (pKa = 4.7), the converted calcium by lactic acid is higher compared to the one by

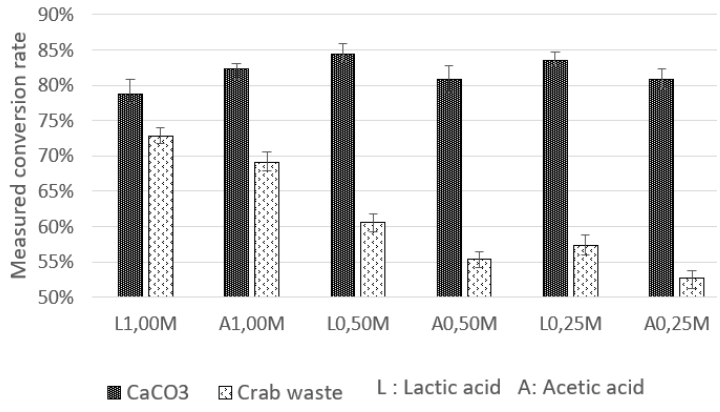

Figure 2 : Measured calcium conversion rate according to the concentration of the acids and the natures of the acids and substrates

acetic acid.

The acid-base reactions present in acid media are presented below:

Lactic acid hydrolysis reaction:

$CH_3CHOHCOOH + H_2O \rightleftarrows CH_3CHOHCOO^- + H_3O^+$

Hydrolysis reaction of acetic acid:

$CH_3COOH + H_2O \rightleftarrows CH_3COO^- + H_3O^+$

Dissolution reaction of calcium carbonate in an acid medium:

$CaCO_3 + 2H_3O^+ \rightleftarrows Ca^{2+} + CO_2 + 3H_2O$

Conversion rates at 1 M concentrations are significantly elevated compared to those at 0.5 M ($p<0.0001)^{\alpha=0,017}$ and 0.25 M ($p<0.0001)^{\alpha=0,017}$. On the other hand, no significant difference ($p=0.471)^{\alpha=0,017}$ was observed between the conversion rates at 0.5 M and 0.25 M. Going deeper in detail, for crude $CaCO_3$, the conversion rates are relatively the same ($p>0.010$ vs. 0.50 M)$^{\alpha=0,003}$. For lactic acid in particular, the conversion rate is lower at 1 M compared to the other concentrations of the same group and amounts to 78.8%±1.8%.

The decrease in the rate of conversion by lactic acid at 1.00 M experimentally measured is due to the low solubility of calcium lactate. Indeed, its conversion rate is equivalent to a concentration of 85 g/l, which corresponds to the solubility of this salt at 30°C (Vavrusova *et al.*, 2014).

For crab wastes, the conversion rates are significantly ($p=0.001$ between 0.50 M and 0.25 M; $p<0.0001$ between 0.50 M and 1.00 M)$^{\alpha=0,003}$ increasing as a function of the concentration.

The increase in the conversion rate as a function of the concentration is due to the fact that a high concentration brings a driving force to the acids so that the diffusion takes place in an optimal manner. These acids then readily diffuse through the protein and chitin layers (Fadli *et al.*, 2018).

Fadli *et al.* (2017) obtained, with shrimp carcasses, increasing conversion rates as a function of HCl concentration: 32%, 93%, 96%, 97% and 98%, respectively for 0.2 M, 0.6 M, 1M, 1.4M and 1.8 M. Jung *et al.* (2005) also obtained similar results. However, the molar ratios between acid and crustaceans in those above-mentioned studies vary with concentration since the amount of crustacean remains the same for all concentrations, causing the acid to become increasingly in excess compared to the minerals. Thus, the reaction is favored in the sense of the dissolution of the calcium carbonate in a too acidic medium.

There are very few studies on the demineralization of crustaceans under stoichiometric conditions. Regis *et al.* (2015) worked on the demineralization of the *Litopenaeus vannamei* shrimp. Acetic acid at 0.48 M under stoichiometric conditions allowed a conversion rate of 83.6%, which is higher than our results obtained under the same conditions. This would be explained by the fact that $CaCO_3$ is more predominant and strongly bound to the chitin embedded in the shell of the crab than $CaCO_3$ in the shell of the shrimp (Gbenebor *et al.*, 2016). Gbenebor *et al.* (2018) obtained conversion rates (32°C) at 1 M hydrochloric acid and acetic acid, respectively of 94.0% and 21.5%, with the crab *Liocarcinus vernalis*. The conversion rate at 1 M acetic acid in our study is 69.1%, higher than their results with acetic acid. The $CaCO_3$-chitin bond would therefore be less strong in Scylla serrata, the species of crab used in our study, compared to that bond in *Liocarcinus vernalis*.

Salt purities

The purities of the salts resulting from the action of lactic and acetic acids on the raw $CaCO_3$ and on the crab wastes were evaluated.

Figure. 3 shows the purities according to the substrate, the reagent and the concentration. It is shown that the solids directly after drying are not entirely composed of salt.

The impurities of the salts would be partly explained by the fact that they are in hydrated form. Indeed, calcium lactate pentahydrate becomes anhydrous between 35 and 135°C (Sakata *et al.*, 2006), calcium acetate at 180-220°C (Asmi and Low, 2014).

Salts from raw $CaCO_3$ are significantly ($p<0.0001)^{\alpha=0,05}$ purer than those from crab wastes. The latter have an orange color and gel after a few minutes in contact with air. The orange color of salts from crabs shows the presence of astaxanthin contained in the shells. Proteins could also be solubilized by acids. Proteins would become positively or negatively charged at pH below or above

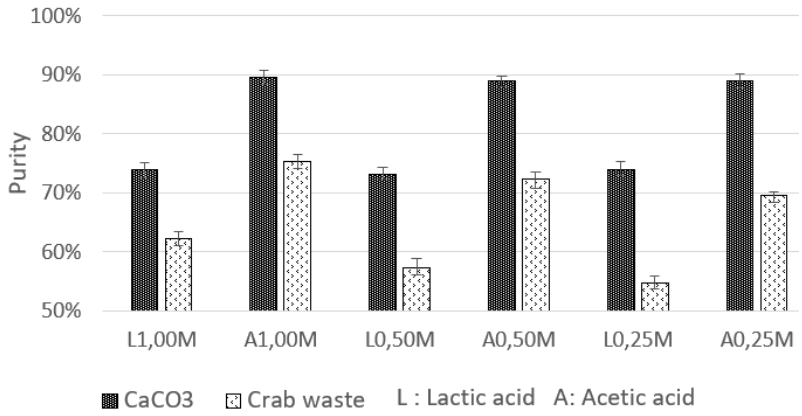

Figure 3: Salt purities according to the concentration of the acids and the natures of the acids and substrates

its isoelectric point, which would increase electrostatic repulsion between protein molecules and hydration of charged residues, causing its solubilization (Chen *et al.*, 2016). In addition, some myofibrillar proteins can also become soluble (Rawdkuen *et al.*, 2009), these proteins play a key role in the gelation of flesh (An *et al.*, 1996). This would explain the gelled appearance of salts from crabs in contact with air.

Salts prepared at 1.00 M are significantly purer than those from salts prepared at 0.50 M ($p<0.0001)^{\alpha=0,017}$ and 0.25 M ($p<0.0001)^{\alpha=0,017}$. On the other hand, no significant difference was observed between the purities of the salts prepared at 0.50 M and 0.25 M ($p=0.026)\alpha=0,017$. However, looking in more detail, the concentration has no influence on the purity of the salts from $CaCO_3$ ($p>0.325$ for all)$^{\alpha=0,003}$. It has a considerable influence ($p=0.001$ between 0.50 M and 0.25 M; $p<0.0001$ between 0.50 M and 1.00 M)$^{\alpha=0,003}$ on the salts from crabs.

Since the rate of conversion of calcium from crab wastes increases with concentration, the salt is much more present in the collected solid for high concentrations.

CONCLUSION

In this study, the conversion of calcium inside the crab wastes into salt was optimized and the purities of the collected salts were evaluated. The experiments showed that crab $CaCO_3$ conversion rates of 74.01±1.11% and 69.14±1.34% respectively with lactic acid and acetic acid could be achieved with purities of 62,14±1,20% and 75,24±1,22% respectively. Calcium salts can therefore be collected and reused during the transformation of crab waste into chitosan, from the first stage of the process: the demineralization. Hence, the environmental impact of the production of chitosan is reduced. Nevertheless, it will be necessary to study the next stages of production of chitosan: the deproteinization and the deacetylation. The efficiency of these two steps could be optimized by varying the concentration of the reagents, the reaction time, the temperature. Moreover, recycling the wastes from these two steps will be needed to reduce even more the environmental impact of the process of chitosan production.

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