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Optimization of Reaction Temperature and Time on the Deproteinization Process during the Extraction of Chitin from Shrimp Shell Waste

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ABSTRACT

Chitin is found in abundance in shrimp shells and is a highly sought component for bioplastic development. In the Philippines, shrimp meat processing discards shrimp shell as wastes which could be valorized by extracting chitin using the chemical method. Extraction of chitin starts with the demineralization of the sample with 1M HCl, at 25°C to 30°C, with a solid to solvent ratio of 1g:10mL for 1 hour. The samples are then processed for deproteinization with 1M NaOH, at 30°C to 70°C, with a solid to solvent ratio of 1g:10mL, for 1-24 hours. This study aims to extract chitin from shrimp shells and to optimize reaction temperature and time of the deproteinization step. The deproteinization temperature (30°C to 70°C) and time (1 hour to 24 hours) were optimized using Response Surface Methodology (RSM) by maximizing the extent of deproteinization (DP). The optimum conditions were determined to be at 53°C and 24 hours, where the overall yield is 19.72% (±0.19) and the DP is 50.79% (±1.33). Lastly, the extracted chitin at the optimum conditions was determined to have a 94.17% (±1.2) degree of acetylation and a molecular weight of 29.15 kDa (±2.39). These are better than the commercial chitin which has a molecular weight requirement of ≥ 5 kDa and a degree of acetylation of ≥ 90%.

Keywords: Chitin extraction; Chitin; Optimization; RSM; Valorization; Waste shrimp shells

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1. Introduction

The Philippines has a large shrimp-based industry with 91 hatcheries and 1,860 farms spread throughout the country [1]. These industries produce a large quantity of shrimp wastes but provide no record on their handling and disposal [2]. This indicates that shrimp shell waste is underutilized as a valuable resource for other product extraction. The underutilization of shrimp wastes poses a problem for these industries due to environmental concerns about its disposal [3]. As such, there is a need to valorize shrimp waste for both environmental and economic reasons to prevent decay and to convert the biomaterial into a valuable product [4].

Shrimp shells are mainly made up of about 25.9% (±2.0) crude protein, 2.4% (±1.0) lipids, 27.9% (±0.9) ash, and 43.8% (±3.9) chitin [5]. The lipids found in shrimp shells are used in food formulations and other industrial products [6]. Protein from the shell can also be extracted and used as a food additive for animal feed [7]. Furthermore, chitin is the most valuable component of shrimp shells, given the numerous applications it has in the fields of medicine, food, agriculture, wastewater treatment, and more [8]. Shrimp shells contain the highest concentration of chitin among other sources like crustaceans and insects [9].

Chitin is also inexpensive since it is waste material from shrimp products that are produced in large quantities; thus, shrimp shells are the main resource for chitin extraction [9-11]. Chitin is present in shrimp shells as a constituent of a complex network with proteins on calcium carbonate and other minerals deposited to form a rigid shell [12]. The shell is made of layers that are divided into three parts, namely the epicuticle, exocuticle, and the endocuticle [13].

In the multilayer cuticle, chitin is found mainly in the inner layer of the cuticle [14]. It is founded in the endocuticle which is heavily calcified and stacked in continuously changing orientation of the lamellae in the form of chitin-protein fiber matrix [13]. The middle layer simply consists of some chitin fiber and minerals while the outer layer is composed of a thin layer of proteins embedded with minerals as shown in Fig. 1 [13, 14].

Extraction of chitin can be done using either the biological or chemical method. However, the chemical method is preferred due to its low cost and its being suitable for mass production, high efficiency, higher purity of chitin extracted, and it is a relatively well-established process compared to the biological method [9, 15–18].

The general steps for the extraction of chitin are as follows: (1) Pre-treatment process which involves the deshelling, washing, drying, and grinding of the shrimp shells to remove any contaminants and remaining muscle particles as well as maintaining a low moisture content (<10%) for its prolonged use [19]. (2) Demineralization involves using an acidic solution for the removal of minerals like CaCO3 and inorganic components, which are embedded between layers of the shell. (3) Deproteination involves the removal of proteins from the material using an alkaline solution. (4) Bleaching involves the removal of the pigments present to produce a commercial grade chitin [7].

Extraction processes present in literature have various process conditions in the deproteinization step. These process conditions are reaction time (1 hour to 24 hours), reaction temperature (20°C to 100°C), and NaOH concentration (0.1M to 2M) [20-

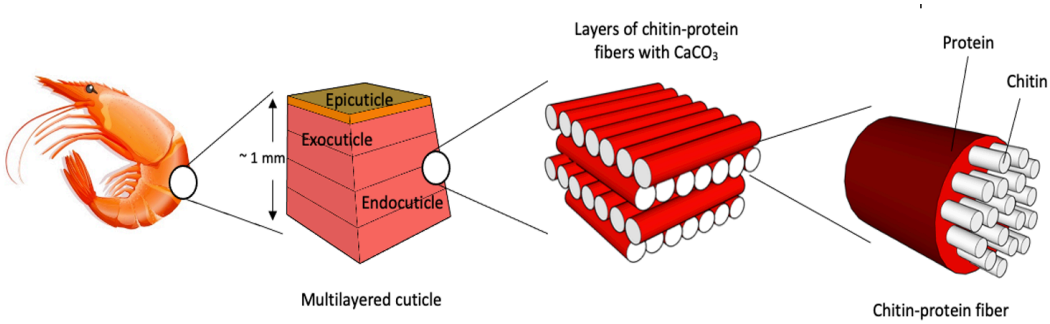

Fig. 1. Layers and structure of shrimp shells [14] (rendered using SketchUp).

32]. In the deproteinization step, it is possible to work with a NaOH concentration of 0.125M to 5.0M (12) and an operating temperature of 50°C to 80°C [33].

The general accepted concentration of the base in the extraction of chitin is 1M [28, 34-35]. Other parameters, such as the solid-to-solvent ratio, have a general accepted value of 1g:10mL from literature [21, 22, 26, 27, 36–39]. As such, there is now the need to optimize the reaction temperature and reaction time from these parameters at constant 1M NaOH and a solid-to-solvent ratio of 1g:10mL

This research aims to help in solving the problem of excessive waste from shrimp production facilities by giving it economic value in terms of chitin extraction. Most studies in literature have used fresh shrimp and little study has been done for the valorization of shrimp shell waste.

2. Materials and Methods

2.1 Materials

Shrimp waste was obtained from a local company in Mandaue City, Cebu. Chemical reagents such as sodium hydroxide (NaOH, 98% w/w), hydrochloric acid (HCl, 37% w/w) of analytical grade and bleach solution (NaOCl, 7% w/w) of technical grade were obtained through local suppliers. All reagents were used without further purification.

2.2 Sample preparation

The shrimp shells were manually removed from its meat and then washed thoroughly in running water to remove loose meat and blood. The samples were then sun-dried until the sample was crisp. The drying process was then finished inside an oven (Memmert, UM400) at 50°C to 70°C until the moisture content of the sample was reduced to <10%.

2.3 Characterization of shrimp shell waste

The samples were characterized in triplicates in terms of their moisture (AOAC 930.15), crude protein (AOAC 2001.11), crude fat (AOAC 920.39), volatile matter (ASTM E897-88), ash (AOAC 942.05), and calcium content (AOAC 927.02). Particle size distribution of the sample was determined using ASTM D452.

2.3.1 Fixed carbon content

Fixed carbon content of the sample was determined by the following empirical formula [40]:

$$\%FC = 100 - \%VM - \%Ash - \%M, \quad (2.1)$$

where %FC is the fixed carbon content, %VM is the volatile matter content, %Ash

is the ash content, and %M is the moisture content.

2.3.2 Chitin content

Chitin content of the sample was determined by the following empirical formula [5]:

$$\%Chitin = 1 - \%CP - \%CF - \%Ash, \quad (2.2)$$

where %CP is the crude protein content, %CF is the crude fat content, and %Chitin is the chitin content.

2.4 Demineralization of dried shrimp shells

From the calcium content of ground shrimp shells (GSS), 1M HCl was the determined concentration required with a solid-to-solvent ratio of 1g:10mL in a beaker with occasional stirring for 60 minutes. The reaction was performed in a water bath to maintain its temperature at 20°C to 25°C. After 60 minutes, the demineralized shrimp shells were filtered until the filtrate registered as neutral. It was then dried until the constant weight of the sample was achieved. The demineralized sample was then characterized by its moisture content, ash, calcium, crude protein, crude fat, volatile matter, fixed carbon, and chitin.

2.5 Design of experiment

The deproteinization step was optimized using design of experiment (DOE) through response surface methodology (RSM). DOE-RSM is a planned approach to determine the cause-and-effect relationship between variables to a response variable in a limited number of experimental runs [41, 42].

An adequate functional relationship between the response (y) and its associated factors (x_i) at the optimum point can be approximated using the second-order model as shown in Eq. (2.3).

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum \sum_{i<j} \beta_{ij} x_i x_j \sum_{i=1}^{k} \beta_{ii} x_i^2 + \varepsilon. \quad (2.3)$$

In the equation, the coefficients, β, are the regression coefficients for the process and ε is the experimental or residual error. The second-order model is a very flexible model which can take a wide variety of functional forms [43]. Therefore, it is a good model to approximate the true response surface.

The central composite design (CCD), a type of RSM, was used to investigate the effects of reaction temperature (x_1) and reaction time (x_2) at constant NaOH concentration and solid-to-solvent ratio, with the response variable being the extent of deproteinization (DP).

The use of CCD allowed the estimation of the nonlinearity of responses in the given data set and determines the maximum information needed from minimum experimental trials [44]. This design consists of a full 2^2 full factorial with one block, no replication for the corner points, two axial points, and one center point.

Using StatEase Design Expert statistical software, a design table was constructed from the specific ranges of reaction temperature and time. For reaction temperature, the ranges for experimentation were from 30°C to 70°C while the reaction time of the deproteinization process ranges from 1 hour to 24 hours.

2.6 Deproteinization of shrimp shells

The deproteinization consisted of 14 batches to be treated separately with the reaction temperature and their corresponding reaction times as presented in Table 1. For each batch, 1.0 M NaOH solution with a solid-to-solvent ratio of 1g:10mL was

Table 1. Experimental design layout and the computed responses.

Factor Levels		Final Protein Contenta (w/w %)	Extent of Deproteinization, (%) Response Variable	Chitin Yield, (%) (Secondary Response Variable)
Temperature ($^{\circ}C$)	Time (hr)			
30	12.5	34.81 ± 0.10	45.13 ± 0.15	20.11 ± 2.88
35.9	20.6	29.57 ± 0.20	56.56 ± 0.30	17.18 ± 1.91
35.9	4.4	36.22 ± 0.38	45.52 ± 0.58	18.71 ± 1.74
64.1	4.4	40.19 ± 0.25	44.13 ± 0.35	18.26 ± 2.50
64.1	20.6	23.03 ± 0.12	56.08 ± 0.22	26.15 ± 0.42
50	12.5	30.78 ± 0.16	55.11 ± 0.23	18.81 ± 2.37
50	1	36.80 ± 0.25	39.20 ± 0.41	21.91 ± 1.84
50	24	27.98 ± 0.33	55.09 ± 0.53	21.38 ± 0.91
50	12.5	32.33 ± 0.45	50.66 ± 0.68	17.9 ± 2.19
50	12.5	28.37 ± 0.27	49.58 ± 0.49	21.83 ± 2.20
50	12.5	29.84 ± 0.10	51.59 ± 0.16	20.04 ± 2.89
50	12.5	32.06 ± 0.12	53.41 ± 0.17	18.66 ± 2.12
50	12.5	34.29 ± 0.17	47.56 ± 0.26	20.12 ± 2.49
70	12.5	33.64 ± 0.49	49.21 ± 0.74	20.08 ± 2.00

added to 50g of demineralized shrimp shells (dry-basis) . Then the sample was extracted in an incubator shaker set at 150 rpm at 30°C to 70°C for 1 hour to 24 hours. After that, they were filtered using a vacuum filter and washed separately with distilled water to remove excess NaOH and soluble proteins. Washing of the samples was stopped when the filtrate registered a neutral pH using a universal pH strip. The batches were then placed in a labelled evaporating dish and were dried to constant weight. Lastly, each batch was then analyzed for its crude protein content.

2.6.1 Extent of deproteinization

The extent of the deproteinization (DP) can be calculated using the following equation [45].

$$DP = \frac{(P_i m_i) - (P_f m_f)}{P_i m_i} \times 100. \quad (2.4)$$

In the equation, P_i and P_f are the protein contents (w/w) before and after the deproteinization reaction, while m_i and m_f are the initial and final mass of the sample (g) in the deproteinization process, respectively.

2.7 Statistical analysis

Optimization and statistical analysis were conducted using StatEase Design-Expert software. Analysis of variance (ANOVA) was performed within the program to evaluate the significance of model terms. The optimization was focused on maximizing the extent of deproteinization by adjusting reaction temperature and time. The model coefficients were derived from the analysis, and the program was used to identify the optimal conditions along with the corresponding predicted extent of deproteinization.

To validate the model, the deproteinization process was repeated under the determined optimal conditions. The experimental results were compared with the model's predicted value with a 5% relative

difference between the two considered acceptable.

2.8 Bleaching of shrimp shells

The remaining deproteinized shrimp shells were then bleached with 7% w/w sodium hypochlorite with a solid-to-solvent ratio of 1:5. These were occasionally held in a vortex shaker to facilitate the bleaching process while maintaining the solution at 20°C-25°C. They were then filtered until neutrality with distilled water. The samples were then dried to a constant weight.

2.8.1 Chitin content analysis after deproteinization

Chitin content of the bleached samples was determined by gravimetric method [46]. Around 0.2g to 0.4g of each of the 14 bleached samples was digested with 100mL of 5% (w/w) NaOH for 1 hour at 105 . These were then filtered until the filtrate is neutral. The sample was then dried in an oven at 50°C - 60°C to constant weight. Each sample was then incinerated in triplicates in the muffle furnace (Vecstar, LF3) at 600°C for 6 hrs. The chitin content was measured in terms of the mass loss during the incineration of the extracted chitin using the following equation.

$$\%Chitin = \frac{m_d - m_a}{m_s} \times 100. \qquad (2.5)$$

In the equation, m_d is the mass of the sample digested, m_a is the mass of the resulting ash sample, and m_s is the mass of the sample prior to the digestion process.

2.9 Physical properties of the chitin sample at the optimum conditions

2.9.1 Molecular weight

The molecular weight of the chitin was determined by the viscometric method [47]. It can be determined by using the Mark-Houwink relationship for the molecular weight and intrinsic viscosity of the sample [48].

$$[\eta] = K \times M^a, \qquad (2.6)$$

where $[\eta]$ is the intrinsic viscosity of the sample (mL/g), which is defined as the ratio of the increase in specific viscosity to concentration when the latter tends towards zero [49].

In addition to this, M is the molecular weight of the sample (Da), K and a are constants depending on the solute-solvent system. Mark-Houwink's constants were experimentally determined based on a known molecular weight of the sample and measured intrinsic viscosity of the sample on a specific solute-solvent system [47].

For a chitin-40% NaOH system, the constants for K and a were 0.10 mL/g and 0.68, respectively [47]. It was prepared by dissolving powdered chitin to the NaOH solution for 72 hours and stored at 4°C using a chiller.

The method was based on the measurement of the flow time, to (s) of the same volume of the 40% NaOH and the chitin-NaOH solution, t(s) using an Ubbelohde viscometer to determine its specific viscosity (unitless). Specific viscosity is simply the ratio of the absolute viscosity of the fluid to that of the reference fluid as mathematically expressed using Eq. (2.7) [50].

$$\eta_{sp} = \frac{t - t_0}{t_0}. \qquad (2.7)$$

Dividing specific viscosity by the concentration of the chitin solution (C_i) in g/mL, gives reduced viscosity (g/mL) [50].

$$\eta_{red} = \frac{\eta_{sp}}{C_i}. \qquad (2.8)$$

2.9.2 Degree of acetylation

The degree of acetylation of the chitin was determined from the absorbance

Table 2. Physicochemical properties of the raw GSS and %reduction due to demineralization.

Composition (w/w%)	Value	
	Raw GSS	%Reduction due to demineralization
Moisture	9.29[c] ± 0.02	-
Asha	45.04 ± 0.11	82.82%
Calcium[a]	16.84 ± 0.05	85.19%
Crude Protein[a]	32.97 ± 0.24	33.32%
Crude Fat[a]	0.47 ± 0.07	100%
Chitin[b]	21.52 ± 0.10	0.64%
Volatile Carbon[a]	40.28 ± 2.10	40.78%
Fixed Carbon[a]	13.31 ± 1.94	40.78%
Particle Size (μm)	348.87	-

[a] dry basis; [b] by calculation; [c] dry sample; [d] wet sample

peaks of chitin from its FTIR result using the following equation [51].

$$\%DA = \frac{A_{1655}}{A_{3450}} \times 115\%. \qquad (2.9)$$

In the equation, A_{1655} is the absorbance of the amide group of the chitin at 1655 cm-1 and A_{3450} is the absorbance of the primary amine group at 3450 cm^{-1} [52].

3. Results and Discussion

3.1 Demineralization

Demineralization's main objective is the removal of minerals, specifically calcium, from the GSS to facilitate efficient chitin extraction. Proximate analysis showed that the GSS is predominantly composed of minerals such as calcium which upon demineralization removes about 82.82% and 85.19%, respectively, as shown in Table 2.

After the demineralization, there are minerals that are still embedded in the GSS matrix. These residual minerals will not hinder the extraction of chitin due to the absence of a heavy calcified layer in the protein-chitin matrix.

On the other hand, analysis also showed that there are other components that were removed during this process.

First, 33.32% of the crude protein was removed from the solid matrix. A similar extent of protein removed during this step is reported in a separate study [53]. This could be due to the denaturation of soluble proteins in acidic conditions [54].

Second is the reduction of chitin content by 0.64% after demineralization process. This is attributed to the deacetylation of chitin during the process which can possibly occur even in a controlled environment [35, 55].

Overall, the demineralization step, aside from its effective reduction of ash and calcium content, also increases the mass ratio of chitin to protein ratio from 0.65 to 0.97. This indicates that the demineralization process is a crucial step in aiding chitin extraction by also removing the protein content in the sample.

3.2 Extent of deproteinization

Demineralization of the GSS removed the heavy calcified layer on the protein-chitin matrix alongside some protein. This completely exposes the protein-chitin matrix during deproteinization. Removal of protein is recorded to be in the ranges of 39% to 56% presented in terms of the extent of deproteinization as shown in Table 1.

3.3 Model fitting and response surface analysis

Statistical analysis using Design-Expert indicated that not all terms significantly contributed to the empirical model. Therefore, the model was refined to include only significant parameters. This was done by evaluation of the probability value (p-value) at a significance level of 0.05. The terms that have a p-value of less than 0.05 ($p < 0.05$) are statistically significant. From the initial empirical model equation determined, the reaction time linear effect ($p = 0.001$) was considered as statistically significant.

Table 3. Regression coefficients and regression statistics of the reduced polynomial model.

Coefficient	Term	Value	Standard Error	t-stat	p
β_0	Intercept	37.41	0.947	53.42	0.000
β_1	x_1	0.0347	0.985	0.50	0.631
β_2	x_2	1.129	0.985	5.77	0.000
β_{22}	x_2x_2	0.0172	1.02	-1.11	0.295
Model summary					
Standard error (S)		Residual squared (R^2)		Adjusted R^2	Predicted R^2
2.78687		79.94%		71.02%	59.01%

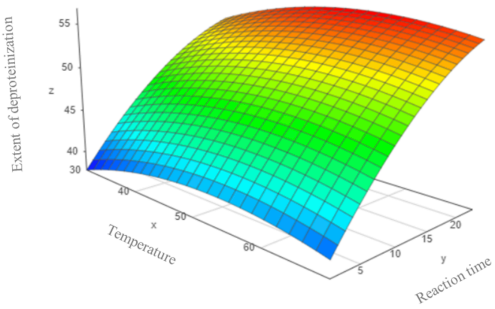

Fig. 2. Surface plot of the extent of deproteinization against reaction time and temperature.

Thus, the model was reduced by removing the temperature-reaction time (x_1x_2) and the temperature-temperature (x_1^2) factors which have the highest p-values of 0.878 and 0.2987, respectively (Table 3). This reduced model yields the following model equation.

$$y = 37.41 + 0.0347x_1 + 1.129x_2 - 0.0172x_2^2. \quad (3.1)$$

The statistical program showed that from this model the optimum conditions were determined to be at 53°C and 24 hours with a predicted extent of deproteinization of 56.94%. The relationship of reaction temperature and time to the extent of deproteinization can be visualized using a surface plot as shown in Fig. 2.

The surface in Fig. 2 showed that temperatures ranging from 40°C to 60°C for more than 20 hours can still achieve greater than 55% of extent of deproteinization. This is expected because deproteinization is a slow process that requires longer reaction time to effectively remove higher protein in the protein-chitin matrix [34].

On the other hand, the surface plot indicates that reaction temperature was optimized while reaction time was not, due to the absence of a concrete peak point.

While it is possible that increasing the temperature beyond 60°C and extending the reaction time past 20 hours would further enhance the deproteinization [56], doing so risks inducing chitin deacetylation. In the presence of NaOH and elevated temperatures, chitin undergoes hydrolysis of the acetamide groups, converting it into chitosan [57, 58]. This is an unwanted conversion because it alters the physiochemical properties and limits the extent of usable chitin in the process.

Reported extent of deproteinization in literature ranges from 82.93% to 99% [21, 24]. This difference can be partially attributed to a key methodological distinction as many literature reports include protein removal during both deproteinization and demineralization steps. This study, on the other hand, specifically evaluates the deproteinization step alone. As a result, the protein removed exclusively in the deproteinization phase is inherently lower.

While the extent of deproteinization achieved in this study is lower compared to other reports, there could be other underlying factors that contribute to this outcome. One key factor is the morphological structure of raw GSS. A stronger chitin-protein matrix may require higher NaOH concentration or a high solid-to-solvent ratio for effective protein removal. Increasing NaOH concentration, however, risks inducing chitin deacetylation which converts it to chitosan [57, 58]. This is supported by a relatively high chitin yield of 17.18% to 26.15% as some reported a low yield in the ranges of 1.99% to 4.92% [27].

3.4 Validation of response model

The predicted extent of deproteinization, calculated from the model, is 56.94% under the identified optimum conditions of 53°C and 24 hours. This represents the central value within the model's prediction interval (PI) of 50.51% to 63.67%. The PI estimates the range within which the actual result is expected to fall, accounting for potential variability within the system.

Experimental results revealed that the extent of deproteinization achieved under these conditions is 50.79% 1.33% which exceeds the 5% relative difference threshold from the predicted value. Experimental extent of deproteinization, however, falls in the lower boundary of the prediction interval of the program which means that the result is still acceptable to the statistical inference made by the program.

The observed discrepancy can be attributed to several factors including the inherent variability in the deproteinization process, potential deviations in experimental execution, and limitations of the empirical model to fully capture complex interactions between variables.

Table 4. The FITR bands of chitin are isolated from shrimp shell wastes at 53°C for 24 hrs in the deproteinization step.

Functional Group	Extracted Chitin (This Study)	Shrimp Chitin (cm^{-1})	
		Kaya et al. [60]	Ibitoye et al. [61]
O-H stretching	3617	3437	3431
N-H stretching	3209-3479	3101 -3259	3105 -3257
C-O secondary amide stretch	1656.38	1654	1653
C-O secondary amide stretch	1602.31	1620	1622
N-H bends, C-N stretch	1538.43	1553	1554
C-0 asymmetric stretch in phase ring	1012.17	1024	1010

3.5 Chitin at optimized conditions

The extracted chitin at the determined optimum conditions was characterized in replicates of its molecular weight, surface functional groups, degree of acetylation, and thermogravimetric analysis (TGA).

3.5.1 Molecular weight

The molecular weight of the extracted chitin was determined to be 29.15 kDa ±2.39. The determined molecular weight of the chitin sample adheres to the molecular weight requirement of > 5 kDa [35].

3.5.2 FTIR

The FTIR analysis showed that a total of 16 peaks were present from all the chitin samples. These 16 peaks from the samples consistently show 3 observed strong peaks which are 1951, 1705 and 1199 cm^{-1}. In fact, these strong peaks indicate that the sample has aromatic combination bands (1951 cm^{-1} and 1705 cm^{-1}) and C-O stretch (1199 cm^{-1}) [59]. The analysis showed that the samples have recorded peaks that are observed in shrimp shell chitin which were summarized in Table 4.

The FTIR confirmed that the extracted sample is characterized as an α-chitin due to its characteristic bands of 1656 and 1602 cm^{-1} [60]. This is due to the band being a characteristic of the primary amide components of the chitin [52]. The presence of these bands supports the presence of chitin in the sample.

3.5.3 Degree of acetylation

The degree of acetylation (DA) of the chitin samples was determined to be 94.17% ±1.21 based on its FTIR results. This indicates that the extracted chitin agrees with the DA requirement of > 90% [35]. This means that the physical characteristics and structure of chitin are well preserved during the extraction process.

3.5.4 Overall yield of Chitin

The overall extraction process produced chitin with an overall yield of 19.72% (±0.19).

3.5.5 Thermogravimetric Analysis (TGA) and Differential Thermal Analysis (DTA)

Thermogravimetric analysis of the extracted chitin sample at the optimum conditions showed two steps. The first curve which occurs at around 325K is attributed to the evaporation of moisture present in the chitin sample. The evaporation is supported by the presence of a cooling effect as detected by the DTA (Fig. 3).

The second curve, which begins at 575K to 725K with 83% mass loss can be attributed to the pyrolysis of the polysaccharide structure [62]. This is due to the accompanying endothermic process which is observed to occur at this temperature range.

Another observation in the TG/DTA diagram showed that at temperatures greater than 700K, significant change is present in the DTA curve. This is attributed to a change in the mechanism in the thermal degradation present in the sample [62].

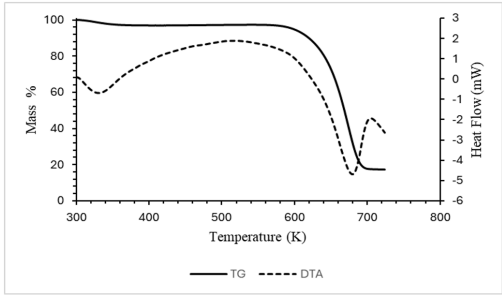

Fig. 3. TG/DTA curve obtained by thermal degradation of Chitin extracted at optimum conditions at a heating rate of 20°C min^{-1}.

4. Conclusions

Chitin has been known to be abundant in crustaceans with extraction possible in waste shrimp shells. These shells are abundant in shrimp processing facilities which separate shrimp shells and meat for exportation. It was determined that demineralization played a vital role in the removal of protein aside from the removal of the calcified layer. The overall chemical extraction process includes demineralization (1M HCl, 1g:10mL, 60 minutes, at room temperature) and deproteinization (1M NaOH, 1g:10mL, 24 hours, at 53°C). The chitin extracted at this condition has a molecular weight of 29.15 kDa (±2.39), degree of acetylation of ≥ 90%, and overall yield of 19.72% (±0.19).

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