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Production and characterisation of chitosan from chitin of snail shells by sequential modification process

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Presently, the snail shells produced wastes that are detrimental to clean immediate environment while over reliance on synthesized polymer are suicidal to environment because they are not degradable. Hence, biological production of polymer through shells of snail recycling will signal an ending to consequences associated with wastes and polymer synthesis. Therefore, the current study is targeted at chitosan extraction, production and characterization from shells of snail. Shells of snail were obtained in Ilesha, Osun State, Nigeria and dried for 24 h in sun and later ground using grinding stone. Extraction of chitin and production of chitosan were both achieved from shells of snail powdery form (10 g). A standard method was employed for determination of physicochemical and functional properties. Chitin and chitosan of 5.50 and 2.46 g were observed with percentages of 55 and 44.73 through decolorization, demineralization, deproteinization and deacetylation (DCMPA) methods. The recorded properties (physicochemical and functional) are: content of nitrogen (1.45%), composition of ash (0.14%), content of moisture (4.84%), viscosity (942cP), solubility (83.65%), deacetylation degree (26.55%), capacity of emulsion (9.54%), density of bulk (0.89 g/ml), capacity of water binding (816.43%) and capacity of fat binding (356.45%). The best and high quality chitosan was produced with references to quantities and properties through DCMPA methods.

Key words: *Chitosan*, chitin, snail shells, decolourization, demenirelaization, deproteinization and deacetylation (DCMPA).

INTRODUCTION

In the modern age, researchers across the globe have shown an interest in issues related to the pollution of our environment. This is connected to various environmental hazards suffered by both animals and humans as a result of this menace. The fallout of various industrial wastes has affected human health as a result of various human

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diseases that arise from decomposing wastes as well as persistent endangerment to land and aquatic animals. The waste generated from snails is also part of the current environmental challenges. The shells of the snails become waste after removal of their inner content, which is beneficial to human health through feeding, but the shells constitute a nuisance to the environment. The waste produced by the fish and fishing industries is high in proteins, fats, minerals, oil, and chitin. These waste materials are starting materials in the industrial production of chitin, chitosan, and other valuable materials.

Waste generated from the shell of snails is the main precursor for the production of chitosan, but there is limited existing work on the use of the aforementioned shell during chitosan production processes. *Athena archatina* (tiger snail), *Archatina fulica* (land snail), *Archachatina marginata* (African giant), *Limcolana aurora* species, and other garden snails are distinct varieties of snails commonly found in the southern part of Nigeria, while the most commonly consumed as a delicacy is *A. fulica,* which belongs to the family Achantinidae (Oluyemisi et al*.,* 2021; Igbinosa et al*.,* 2016). The presence of a high content of carbohydrates (86.83/100 g–92.76/100 g) in snail shell is responsible for the high yield of chitosan in snail shell compared to the amount recorded in seafood waste (Ademolu et al*.,* 2018). The shell of crustaceans is one of the prominent sources of chitin and chitosan because of the biomaterial in abundance and the extraction process on a well-established industrial scale.

The environmental pollution caused by snail shells was caused by the fact that snails are abundant during the rainy season (Ademolu et al., 2018). Restaurants, eateries, hotels, and club houses are the sources of discarded shells of snails that are responsible for a severe degree of threat to the environment (Oluyemisi et al*.,* 2021). After removal of the edible portion of the snail, the shells are indiscriminately abandoned without proper disposal. In 2017, the waste generated from crustacean shells was 8.4 million tonnes (FAO, 2019). As a result of large amounts of shells littering the environment, they have become a nuisance to our immediate environment. Therefore, in a bid to have a clean environment and also to have an economy with massive and immense prosperity, there is an urgent need to recycle, and the process of recycling results in the production of chitosan through chemical deacetylation of earlier formed chitin (Amoo et al*.,* 2019; Kolawole et al*.,* 2017).

The bodies of arthropods are covered by chitin, while the cell walls of mushrooms are majorly made up of chitin. The structural content of algae, coral, and nematodes also contains chitin (Daniel and James, 2019). Chitin exists primarily in nature as cellulose, with applications spanning medicine, agriculture, cosmetics, biotechnology, and scientific bio-inspired materials (Oyekunle and Omoleye, 2019a). Chitins are found in various degrees of acetylation that differ from fully to total acetylated and deacetylated, respectively. Based on its physical property effects, the degree of acetylation is a very important chitin property (Daniel and James, 2019).

The shells of crabs, shrimps, and prawns are a source of organic polysaccharide referred to as chitosan (Ghanaam et al*.,* 2016; Majekodunmi et al*.,* 2017). Chitosan produced through chitin deacetylation is a high molecular weight molecule with biodegradable polymer and also consists of-(14)-2-amino, 2-deoxy-Dglucopyranose (Islam et al*.,* 2017). Chitosan is the most common derivative of chitin formed based on chitin partial Ndeacetylation through modifications of chemical processes with more than one soluble analog. As a result of numerous advantages (non-toxicity, biocompatibility, biodegradability, and non-antigenicity) attributed to chitin and chitosan, they have continued to attract interest globally (Daniel and James, 2019). The quantity of the group of acetyl-glucosamine contained in bio-polymer is a yardstick to differentiate chitin from its derivative (chitosan). Chitin and chitosan are both chemically and thermally difficult to degrade but are highly stable, non-toxic, and biodegradable polymers (Amoo et al*.,* 2019).

De-proteinated and demineralized marine chitosan is a source of chitosan that can be used to make artificial organs, drug membranes, and fibers for use in medicine; fungicides and drug carriers for use in pharmacology; preservatives, coatings, antimicrobial, and antioxidant agents for use in food systems; and body creams, hair additives, and lotions for use in cosmetics (Huang et al*.,* 2020; Barbosa et al., 2020; Barbosa et al., 2020). The food industry has explored and adopted chitosan as a biopolymer as an emulsifier, stabilizer, antibacterial, and thickening agent (Oyekunle, 2019). Pharmaceuticals, tissue engineering, waste water treatment, biotechnology, cosmetics, and food processing are other areas where chitosan is used (Aranaz et al*.,* 2018; Harkin et al*.,* 2019).

Dyes removal, polychlorinated biphenyl (PCB) removal, and chemical waste detoxification are environmental studies where chitin and chitosan are most significantly applied (Kyzas et al*.,* 2017). Chitin and chitosan also find applications in water treatment, such as filtration, desalination, and flocculation/coagulation, which are fields in water treatment where chitosan is useful (Al-Manhel et al*.,* 2018; Raeiatbin and Acikel, 2017). The application of chitosan in tissue engineering is based on stiffness as a result of high crystallinity, which is the brain behind higher mechanical strength (Balagangadharan et al*.,* 2017).

As a result of its exceptional ability to remove surfactants, pesticides, phenol, and polychlorinated biphenyls from wastewater, chitosan is used in wastewater purification as flocculating, coagulating, and chelating agents (Bello and Olafadehan, 2021). Chitosan's use in numerous industrial applications is associated with qualities like biocompatibility, non-toxicity, and adaptability (Antoniraj et al., 2020; Bharathi et al., 2020). Chitin and chitosan derivatives had a global market value of US

$2900 million in 2017, with a CAGR of 14.8% and a forecast of US $63 billion by 2024 (Oyatogun et al., 2020). Furthermore, the chitosan market was estimated to be worth $6.8 billion in 2019 and was projected to grow at a CAGR of 24.7% from 2020 to 2027, based on revenue (as published by Grand View Research in March 2020).

Overreliance on non-biodegradability and enormous space-occupant synthetic polymers constitute challenges linked to environmental disposal. As a result, there has been an increase in research into replacing synthesized polymers with biocompatibility, biodegradability, and non-toxicity biopolymers (Bello and Olafadehan, 2021). The environmental problems caused by synthetic polymers can be tackled through the production of a biopolymer-chitosan from shell wastes of crustaceans (Bello and Olafadehan, 2021). Also, wastes generated by arthropods and pisces by the seafood industries produce offensive odours from dumpsites, displeasing the environment non-aesthetically, thereby resulting in health and environmental hazard (Bello and Olafadehan, 2021). To end this challenge, viable products are produced through the recycling of shell wastes by initial chitin production (chemical processes of demineralization and deproteinization) and subsequent chitosan formation (deacetylation) (Bello and Olafadehan, 2021).

Despite the array of work done on chitosan production from different sources, there are limited or no studies on concurrent production as well as characterization of chitosan from snail shells. Also, the production of chitosan involves four sequential preparation processes; the steps involved can be interchanged in a bid to enhance the quantity and quality of chitosan produced. There is a little work on the production of chitosan through the alteration production process, while in most cases; characterization of produced chitosan obtained through the interchange of production processes has not been investigated. This study focuses on the production and characterization of chitosan. This study is aimed at investigating and determining the physical and chemical properties of chitosan produced from snail shells by the alteration of four production processes. The specific objectives include the preparation of chitin and chitosan from the raw samples of snail shells; extraction and quantification of chitosan from the chitin of snail shells; characterization of chitosan extracted from the chitin of snail shells.

METHODOLOGY

Shells of snail were collected within Esa-Oke community in Osun-State. The place lies in 7.75833°N latitude and 4.89722°E longitude, respectively.

Sample preparation

Shells of snail were subjected to washing, drying (overnight at 50°C) and grinding before storing in a dry place before extraction processes. Chemicals used were of standard analytical grade. Alteration of four order preparation sequential processes was used adopted for five chitosans made from shells of snail (DCMPA, DMCPA, DMPCA, DMPAC, and DPMCA). Traditionally, DPMCA was used as control for processing methods.

DCMPA- decolorization + demineralization + deproteinization + deacetylation
DMCPA- demineralization + decolorization + deproteinization + deacetylation
DMPCA- demineralization + deproteinization + deacetylation + decolorization
DMPAC- demineralization + deproteinization + deacetylation + decolorization
DPMCA- deproteinization + demineralization + decolorization + deacetylation

Extraction of chitosan

Deproteinization

Solution (2.0%) of potassium hydroxide was employed for treatment of shells obtained from snails. This was achieved at 1:20 (w/v) ratio which involved groundshells and solution. The protein was removed from mixtures at 90°C through stirring constantly for 2 h. This was preceded by filtration of sample in a vacuum as well as washing of filtrates through running water from tap for period of 30 min in order to obtain neutral pH of 7. Then, drying of shells of snail that was deproteinized was conducted at 60°C for 24 h in an oven (Shahidi and Synowiecki, 1991).

Demineralization

This was carried out on shells of snail deproteinized using 2.5% in weight per volume of hydrochloric acid for 6 h at room temperature in order to remove the content of mineral at ratio of shells ground to that of solution (1:20 weight/volume). Filtration of sample was done under a vacuum while washing was achieved in a water tap for 30 min until attainment of neutrality pH of 7. Drying of shells demineralized was successful in an oven for period of 20 h at sixty degree centigrade (Shahidi and Synowiecki, 1991).

Decolouration and dewatering

This was done through samples treatment with acetone for 10 min while they were dried at ambient temperature for 2 h with removal of residues formed. The colour-removed snail shells were subjected to washing in tap running with water. This was followed by rinsing, filtering and drying in oven for 24 h at 60°C so as to obtain chitin of snail shells (Shahidi and Synowiecki, 1991).

Deacetylation of chitin

The described method of Yen et al. (2009) was employed in carrying out chitin acetylation removal. The ratio of 1:15 (w/v) for chitin to the solution was adopted for treatment of chitin with 40% sodium hydroxide aqueous solution for 2 h at 105°C. The filtration of chitin was carried out through pumping filter while washing was achieved with deionized water until neutral pH was obtained in order for chitosan to be recorded. The observed chitosan undergo drying in an oven for 24 h at 60°C.

Characterization of chitosan

The yield

The comparison of raw materials measurement weight with the recorded weight of chitosan after treatment was used for chitosan yield determination (Nouri et al., 2016).

A yield was calculated as follows:

$$\text{Chitin yield (\%)} = \frac{(\text{Extracted chitin})\,(g)}{\text{Grinded Snail shells}\,(g))} \times 100 \quad (1)$$

$$\text{Chitosan yield (\%)} = \frac{(\text{Extracted chitosan}\,(g)}{\text{Extracted chitin}\,(g))} \times 100$$

Moisture, ash and nitrogen contents

The content of moisture for chitosan was evaluated through methods of gravimetric (Mohan et al*.,* 2019). The dehydration of sample was carried out in air hot oven for 2 h for constant weight. The percentage of moisture was determined through differences in weights of both wet and that of samples dried in an oven. The procedure previously described by AOAC (1990) was used for determination of content of nitrogen present in chitosan. Initially heated furnace of muffle type at 600°C for period of 8 h was employed for estimation of chitosan ash content. The desiccator was used for cooling of sample and finally weighed to observed ash content (Mohan et al., 2019).

Determination of degree of deacetylation (DD)

This was conducted using modified titration direct methods described by Kjartansson (2008). 0.1 g samples of chitosan were dissolved in hydrochloric acid (0.06 molarity) of 25 mL at normal room temperature for 1h. Titration was done through sodium hydroxide of 0.1 N until regularly stirring was exhibited to obtain 3.75 pH after initial dilution of solution to 50 mm. At 3.75 pH, sodium hydroxide volume was acquired and as well recorded. There was continuous of titration at pH 8 and this was followed by final recording of sodium hydroxide (0.1 M) total volume. The calculation of deacetylation degree was estimated with the following equation.

The volume of NaOH at pH 3.75 was acquired and recorded. Titration was continued to pH 8, and the total volume of NaOH (0.1 M) was recorded. The degree of deacetylation was then calculated using the following equation:

$$\text{Deacetylation Degree} = \frac{(161.16*(V2-V1)N}{W1}$$

Mass of monomer of chistosan is 161.16.

The used solution of sodium hydroxide volume stands for V1 and V2, *N* represented solution of sodium hydroxide (0.1 M) strength and W1 is sample mass immediately after moisture correction. The samples deacetylation degree (DD) was done in triplicate.

Water binding capacity

The method described by Ocloo et al*.* (2011) was adopted for measuring of capacity of water binding. Tube of centrifuge was used for weighing of 0.5 g of chitosan and this was followed by addition of 10 ml of water from distiller. In a bid to ensure that chitosan dissolved prior to period of 30 min at temperature of ambient, there was mixture vortexing for 1 min. For second of five at every 10 min before centrifugation for 25 min at 3,200 rpm, there was shaken of tube. After decantation of supernatant, again the tube was weighed. The following equation was used for calculation of capacity of water binding:

$$\text{Capacity of water binding (\%)} = \frac{\text{Water bound}\,(g)}{\text{Sample weight}\,(g))} \times 100$$

Fat binding capacity

This was achieved using described method by Ocloo et al*.* (2011). Centrifugation tube was used for weighing of 0.5 g of chitosan before addition of distilled 10 mL of water. Vortexing of mixture was done for a minute so as to ensure that chitosan dissolved prior to 30 min in an ambient temperature while at interval of 10 min for 5 s the tube was shaken before centrifugation at 3,200 rpm for 25 min. There was weighing of tube after decantation of supernatant. Through the following equation capacity of water binding was estimated:

$$\text{Capacity of fat binding (\%)} = \frac{Fat\ bound\,(g)}{Sample\ weight\,(g))} \times 100$$

Solubility

The method described by Fernandez-Kim (2004) was utilized for determination of solubility of chitosan from snail shells. The powdery form of chitosan (0.1 g in triplicate) was placed in a tube of centrifuge machine after which 10 mm of 1% of acetic acid was added for period of 30 min so as to dissolve. This was carried out in shaker (incubator) working at 240 rpm as well as 25°C. This was preceded by immersing of solution in water bath containing boiling water for period of 10 min and finally cooled at 25°C before 10 min centrifugation at 10,000 rpm. Decantation of supernatant was followed while particles yet to be dissolved were subjected to washing through 25 mL of water that undergo distillation and centrifugation was achieved at 10,000 rpm. There was removal of supernatant while drying of undissolved pellets was successful at 60°C for 24 h. The percentage of solubility of chitosan was known after weighing of particles. Chitosan solubility was estimated by employing the equation:

Solubility (%) = (Initial weight of tube + chitosan) (Final weight of tube + chitosan) × 100 / (Initial weight of tube + chitosan) – (Initial weight of tube)

Viscosity

This was assessed by method described by Ocloo et al*.* (2011). There was dilution of 1% of acetic acid at 1% concentration on a dry basis with chitosan extracted. The Brookfield viscometer of number two spindle at 50 rpm as well as 25°C with reported values in units of centipoises was employed for determination of viscosity of extracted chitosan from snail shells.

Emulsifying capacity

This was determined through method of Yasumatu et al*.* (1972). 1 g of each of the sample together with distilled cold water of 50 mL as

Plate 1. Sample of washed and sun dried Snail shells obtained from Esa-Oke.
Source: Author

well as 50 mL oil of sun flower were utilized for the preparation of emulsion. There was dispersion of sample of gelatin with a homogenizer/blender. To 50 ml tubes of centrifuges, there was equal distribution of each blended sample into it. There was direct centrifugation of a tube of centrifuge for 10 min at 4000 g while the rest will pass through centrifuged in similar conditions after undergoing heating at 80°C for period of 30 min in a water bath and finally cool in a room temperature. The capacity of emulsification was assessed through the height of layer emulsified as total height of percentage of material in the unheated tubes.

Statistical analysis

The observed data in triplicate were analyzed through appropriate statistics.

RESULTS AND DISCUSSION

Snail shells collection and grinding

Plate 1 illustrates a pictorial diagram of collected shells of snail washed and sun-dried. Grinding stone was used for turning the snail shells into powdery form (Plate 2).

Sequential process modifications on quantity and yield of chitin and chitosan produced from snail shells

The alteration of the sequence of four order involved in process of preparation was adopted for various chitins denoted as DPMCA, DMCPA, DMPCA, and DCMPA (Figure 1a). DPMCA represented steps of sequences for deproteinization, demineralization, decolorization, and deacetylation. DPMCA denotes the process of traditional methods and was chosen as the control for the sample. The amount of the produced chitin is based on methods employed for chitin extraction as DCMPA produced highest quantity of chitin (from 10 g of ground shells of snail, 5.50 g of chitin was recorded) while the least amount was observed in DMPAC (10 g of powdery snail shells produced 4.15 of chitin) (Figure 1a). The highest percentage yield of chitin (55.00) was also found in sequence of extraction involving DCMPA but with DMPAC as process of production methods, the lowest percentage yield of chitin (41.5) was observed (Figure 1b).

The optimum amount of chitosan (chitosan of 2.46 g was obtained from chitin of 5.50 g) found in the current study was recorded through process of production order by DCMPA while least amount (4.15 g of chitin produced 1.30 g of chitosan) was detected through DMPAC sequential method (Figure 2a). The production order steps of DCMPA and DMPAC produced highest and lowest (44.73 and 31.33%) chitosan yields (Figure 2b).

The aforementioned result could be due to fact that there will be slight increment in produced chitin and chitosan when demineralization and deproteinization processes came behind decolorization and dacetylation in order of production steps. Similarly, Adekanmi et al. (2020a) reported the chitin production of 7.24 and 3.55 g while chitosan produced were 60.33 and 49.03% through methods of DCMPA during various protocols of process order for chitosan extraction, quantification as well as characterization from fish (cray). In related scenario, Majekodunmi et al. (2017) reported 51.8 and 43.8 as percentages of yields of chitosan from *Mytilus edulis* as well as *Laevicardium attenuatum*. Gaikwad et al. (2015)

Plate 2. Ground powdery form of snail shell.
Source; Author

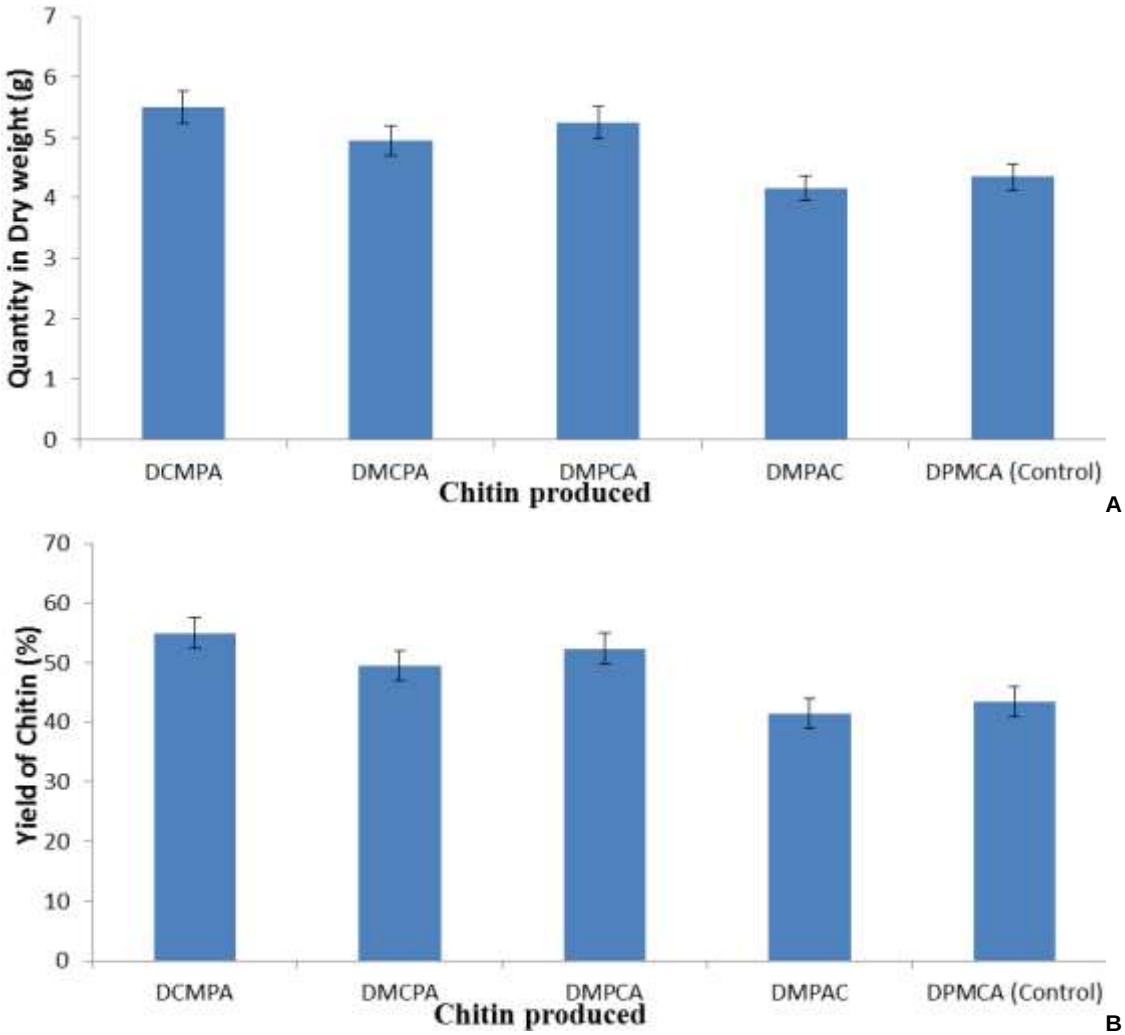

Figure 1. (a) Chitin quantity observed from snail shells through modified production steps. **(b)** Percentage of chitin yields from shells of snail by altered processing methods.
Source: Author

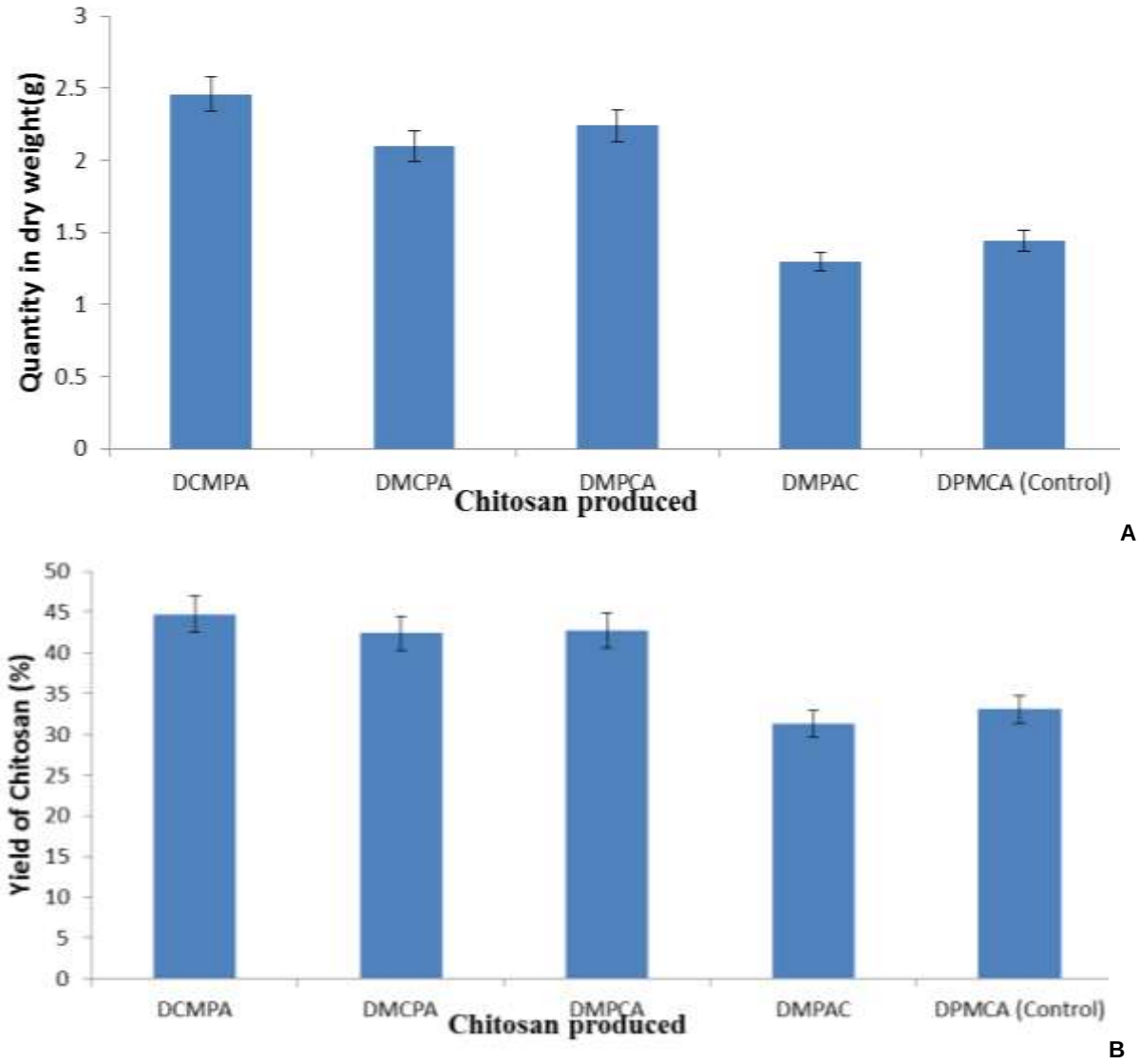

Figure 2. (a) Chitosan quantity produced from shells of snail by step modification process. (**b)** Percentage of chitosan yields from shells of snail through altered production steps.
Source: Author

also observed 53, 49, 52, 41, and 42% yields of chitosan from shells of crabs (*Scylla serrata*) through adoption of various sequential chemical processes (DCMPA, DMCPA, DMPCA, DMPAC, and DPMCA).

Characterization of chitosan produced by sequential production process

Ash content

The measurement of content of ash in chitosan is an indicator of step of dimineralisation effectiveness for calcium carbonate removal. It is expected that quality high grade of chitosan should have content of ash below 1% (No et al*.*, 1995). In respect of the source (Abdou et al., 2008), crustaceans exoskeletons contain large content of calcium carbonate. The low level of viscosity was as a result of impact of qualities of final product particularly solubility on residual content of ash in chitosan. An excellent low content of ash ranging between 0.14 and 0.75% was recorded for content of ash in chitosan produced in this study (Figure 3). This might be connected to effectiveness of steps of demineralization in minerals removal from shells of snail. In a similar study, 0.17% content of ash was observed in chitosan produced from scales of fish through demineralization, deproteinization as well as deacetylation, and decolorization sequential (Adekanmi et al*.*, 2020b). The current study is also in line with Tajik et al*.* (2008) work

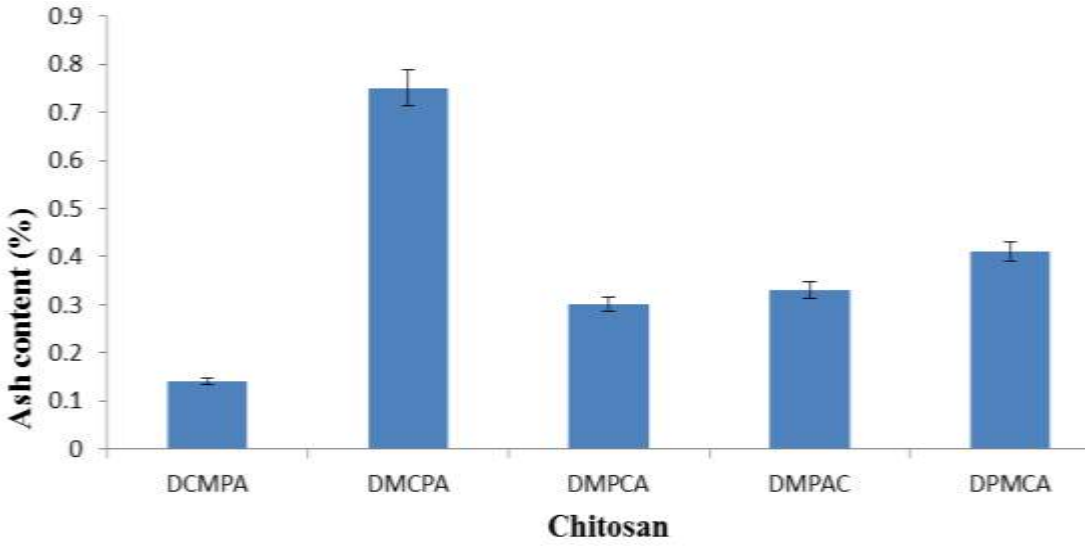

Figure 3. Percentage content of ash in chitosan produced from shells of snail by various modified available process.
Source: Author

where 0.19 and 0.51% were recorded for content of ash in chitosan with adoption of sequential process of production. Also, chitosan produced from shells of cray fish recorded low ash content of 0.12% through production step involving that is not limited to decolorization, demineralization but also deproteinization and deacetylation (Adekanmi et al*.,* 2020a).

Moisture

The content of moisture recorded in this study revealed a slight variation with significant differences of between 2.57 and 5.25 ranges for produced chitosan from shells of snail (Figure 4). Low content of moisture observed in this work is of advantageous due to effects of adsorption of moisture on chitosan capacity of water holding in respect to many industrial applications and processing (Chandumpai et al*.,* 2004). In similar vein, 4.46% moisture content was found during chitosan production and characterization from shells of cray fish with decolorization, demineralization as well deproteinization, and deacetylation as order employed during production procedures. This result is related to the work of Majekodunmi et al*.* (2017), in which 3.28 and 3.84% were found as content of moisture in chitosan produced from both *M. edulis* and *L. attenuatum,* respectively.

Also, significant differences were found in moisture percentages (2.37 and 5.4%) between five chitosan produced from shells of crab (Gaikwad et al*.,* 2015). Concordantly, no significant difference in quantity of moisture (1.0-1.3%) was observed among four chitosan produced from *Artemia.*

Nitrogen content

The percentage content of nitrogen in chitosan found in this work is between the range of 1.24 and 1.55% (Figure 5). This present work is related to the report of Gaikwad et al*.* (2015) where they recorded range of 0.9 and 1.91 as percentages content of nitrogen for produced chitosan. In another closely related manner, Adekanmi et al*.* (2020a) observed 1.25% as content of nitrogen from chitosan formed from shells of cray fish by utilizing decolorization, demineralization, deproteinization, and deacetylation (DCMPA) methods of sequences. Contrary to the present study, the values of 7.06 to 7.97% and 7.32 to 7.51% were reported as percentages of content of nitrogen for chitosan produced from different sources.

Solubility

Excellent and perfect solubility was observed in the current study, with values ranging from 88.16 to 89.20%. The highest percentage values for solubility was found through DMPCA as methods of production while with DPMCA sequence of production, lowest solubility chitosan percentage was recorded (Figure 6). The highest solubility observed in this work shows protein total removal while partial or incomplete removal of protein occurred as a result of low values of solubility in chitosan (Brine and Austin, 1981). The current work is similar to the finding of Gaikwad et al*.* (2015) where significant differences were found through demonstration of an excellent values with range from 81.78 to 88.78% and DCMPA as method of production sequential

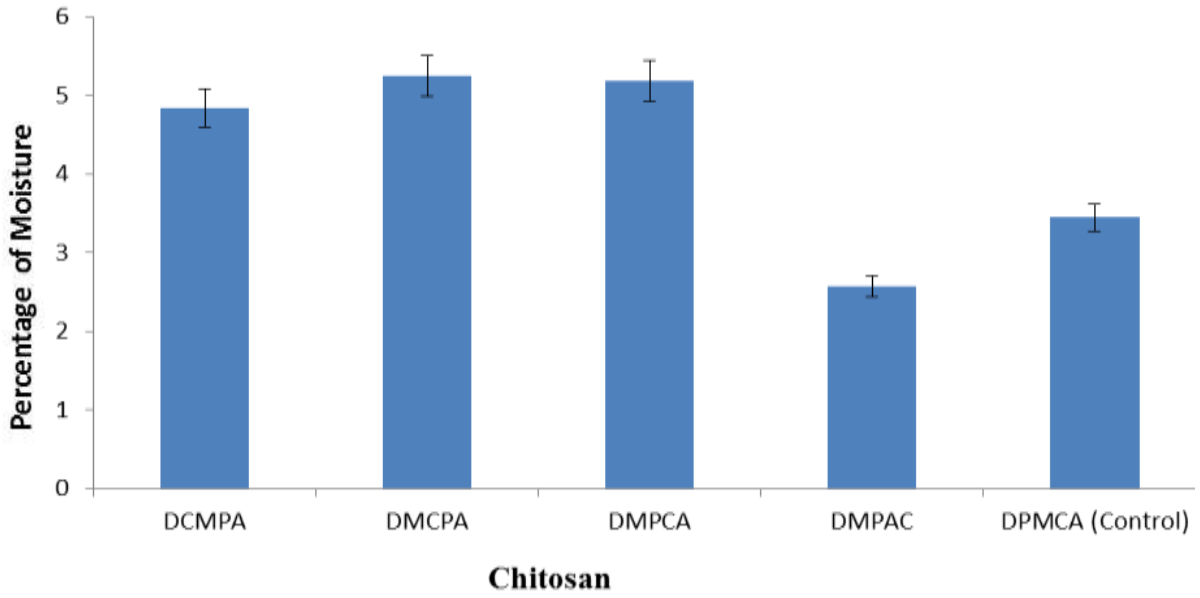

Figure 4. Percentage content of moisture in chitosan observed from shells of snail with sequential different production steps.
Source: Author

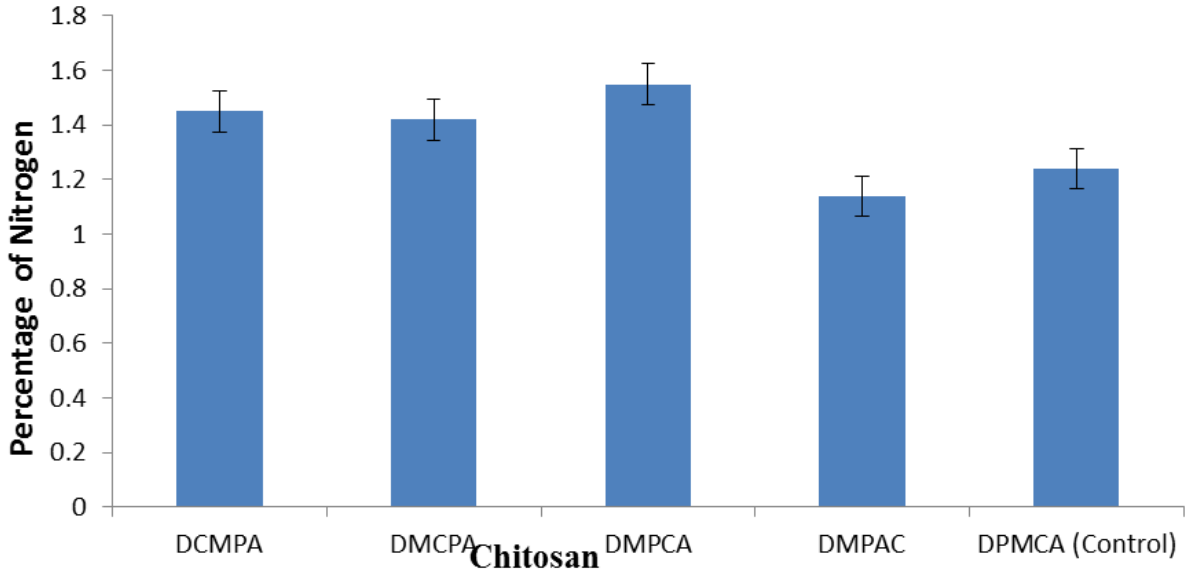

Figure 5. Percentage of content of nitrogen in chitosan produced from shells of snail with various production methods.
Source: Author

recorded lower value ((81.78%) for solubility of chitosan produced from crab samples. The reported solubility in this work is in the same page with values of 88.5% of solubility reported from chitosan produced from shells of cray fish through described sequence of production decolorization, demineralization, deproteinization, and deacetylation methods (Adekanmi et al., 2020a).

Degree of deacetylation

The solubility of chitosan, reactivity of chemical and degradation through biological means are affected by deacetylation degree. The physicochemical and biological properties are also affected by degree of deacetylation (Kumari et al., 2017). The deacetylation degree may

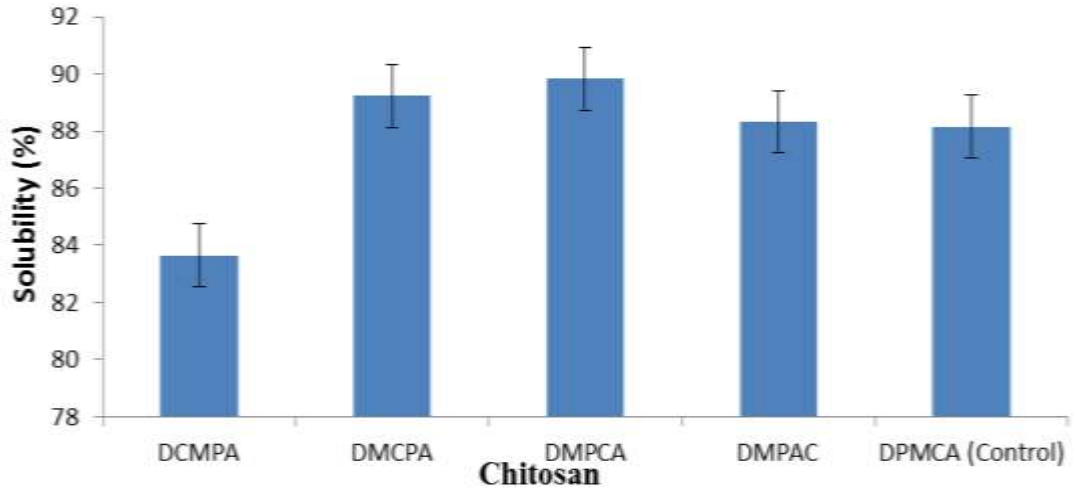

Figure 6. Percentage of solubility of chitosan from shells of snails with varying methods of production.
Source: Author

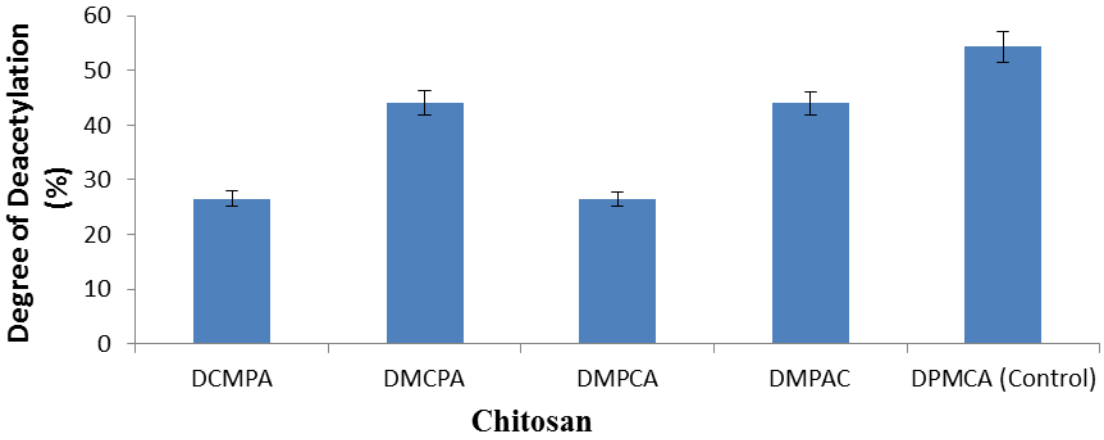

Figure 7. Percentage of deacetylation degree of chitosan from shells of snail with varying sequences of production.
Source: Author

range from 30 to 95% but it dependent on sources of chitosan and procedure or preparation steps (Martino et al*.,* 2005). All chitosans produced in this study had more than 70% of deacetylation degree except 54.2% recorded through DMPCA production sequence (Figure 7). This was similar to a reported 65.33 percentage of deacetylation degree observed from chitosan production from fish scales through methods of DMPAC (Adekanmi et al*.,* 2020b).

Emulsion capacity

The capacity of emulsion for chitosan obtained from shells of snail in this study is between the ranges of 4.26 to 11.04.21% (Figure 8). The least capacity of emulsion (4.26%) was found through DMPAC as production method while the highest value of 11.04% was found in DMPCA method (Figure 8). It was reported that deacetylation degree is a determinant factor in chitosan emulsifying properties; chitosan became emulsifier with low effectiveness through intermediate while poor emulsification was recorded with high deacetylation degree (Del Blanco et al., 1999). In this study, despite 26.43 to 54.28% range of deacetylation degree recorded, they still influence capacity of emulsion. Similarly, 9.33% of capacity of emulsion was found during chitosan extraction, quantification as well as characterization from

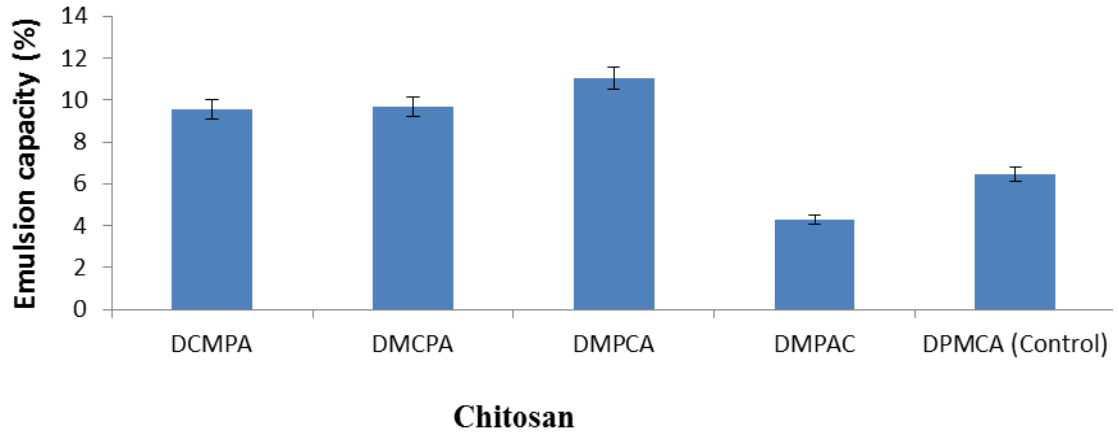

Figure 8. Capacity of emlusion of chitosan produced from shells of snail with modified different sequential process.
Source: Author

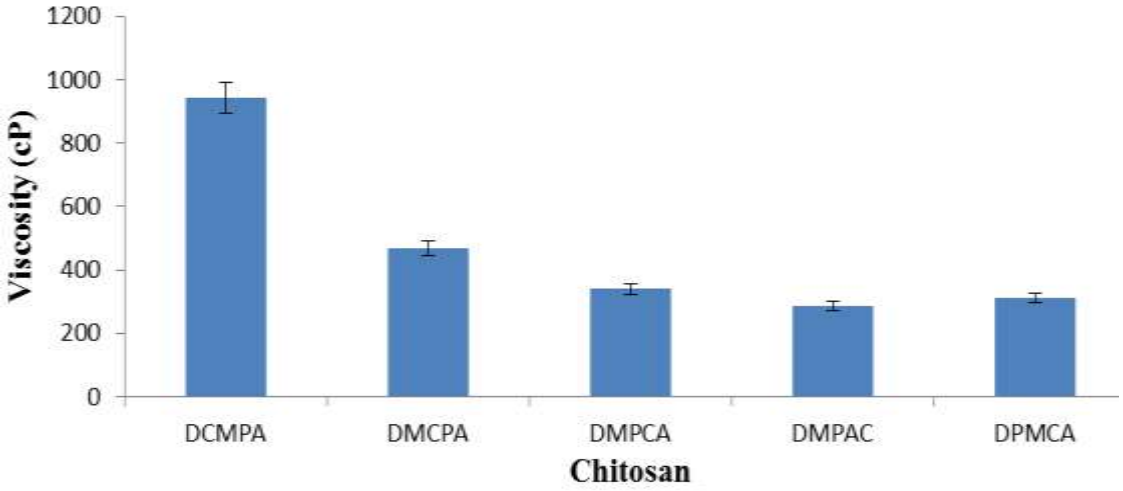

Figure 9. Viscosity of chitosan produced from shells of snail with varying modified sequences.
Source: Author

shells of cray fish (Adekanmi et al*.,* 2020a).

Viscosity

DCMPA had the highest value of viscosity (942 cP) in this study while the lowest value (286 cP), of viscosity was observed through DMPAC method. This is an indication of reduction in level of molecular weight (Figure 9). This was related to the work of No and Meyers (1995) in which species and methods of preparation determine variation in values (from 60 to 5110 cP) of viscosity recorded for produced chitosans. In this work, viscosity of sample produced through DCMPA (942 cP) and other method of sequences adopted showed differences significantly (Figure 9). There was two and three folds increase in viscosity observed with DCMPA than DMCPA (468 cP) and DPMCA (312 cP) methods. The chitosan found in shells of snail had higher viscosity compare to other crustaceans (Tharanathan and Kittur, 2003). Application of chitosan as both thickening and suspending agents in medical fields, cosmetics production and food industry was as a result of higher viscosity but aforementioned applications functions in opposite views with chitosan of low viscosity. In similar page, 720 cP high viscosity was obtained from chitosan extracted, quantified and characterized from shells of crayfish by varied sequential methods (Adekanmi et al*.,* 2020a).

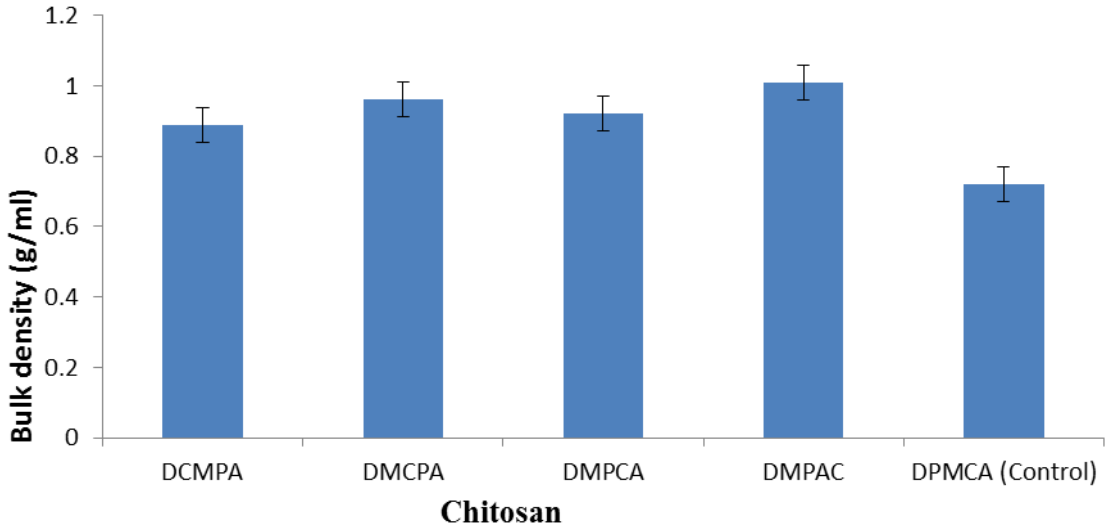

Figure 10. Density of bulk for chitosan produced from shells of snail with modified different sequence processes.
Source: Author

Bulk density

Crayfish, commercial chitin and chitosan bulk density variation was as a result of sources or species where chitosan was produced and preparation methods (Cho et al*.,* 1998). In the above study, the density of bulk for chitosan from shells of snail range from 0.72 to1.01 g/ml (Figure 10). The chitosan from shells of snail had highest density of bulk (1.01 g/ml) through DMPAC method (Figure 10). Low bulk density reveals more porosity of chitosan and effect of treatment with low alkali during deproteiniation (Cho and No, 1999). In a similar development, a chitosan from scales of fish had 1.04 g/ml as density of bulk (Adekanmi et al*.,* 2020b) while 0.87 g/ml was also reported as bulk density from chitosan produced from shells of crayfish (Adekanmi et al*.,* 2020a).

Water binding capacity (WBC)

The values of water binding capacity between 685 and 816% were reported for all five chitosans produced in this study (Figure 11). In agreement with the current work is values recorded by Rout (2011) where binding capacity of water for chitosan vary between 581 and 1150% with 702% as an average (Rout, 2011). In the same vein, similar values were reported by Cho et al. (1998) unlike No et al*.* (2003) that had values with range of 355 to 611% for binding capacity of water. The method of DCMPA had highest water binding capacity and this was preceded by DMPCA, DMCPA, DPMCA, and DMPAC methods with values of 787, 776, 737, and 685%, respectively (Figure 11). As revealed in Figure 11, steps of sequence alteration had immense effects on binding capacity of water. When demineralization was done before deproteinization and deacetylation, an increase in water binding capacity was observed but this is not situation where deproteinization was conducted ahead of demineralization and deacetylation.

In a related finding, Adekanmi et al*.* (2020b) found 682% as binding water capacity for chitosan from scales of fish while 716.33% was also observed for capacity of binding water in shells of crayfish chitosan (Adekanmi et al*.,* 2020a). A decline in water binding capacity was attributed to decoloration process but this is not the case for chitosan from crawfish without bleaching process (Rout, 2001). The functional characteristics of chitin and chitosan were reported to be influenced by physicochemical properties and this varies with sources of species and methods of preparation (No et al., 2003). Crystalline dissimilarities, variation in the quantity of groups forming salts and the products residual content of protein were responsible for possibility of variation in binding capacity of water between polymers of chitin (Knorr, 1982).

Fat binding capacity (FBC)

The chitosan binding capacity of fat for the five shells of snail was evaluated through the use of olive oil. As indicated in Figure 12, the range of 274 and 395% was observed for fat binding capacity in this study. A slightly similar work was reported by Cho et al. (1998) while Li et al*.* (1992) also reported values of range between 217 and 403%. The current work is in line with the reported fat binding capacity (363.33%) observed during characterization of chitosan formed from fish scales

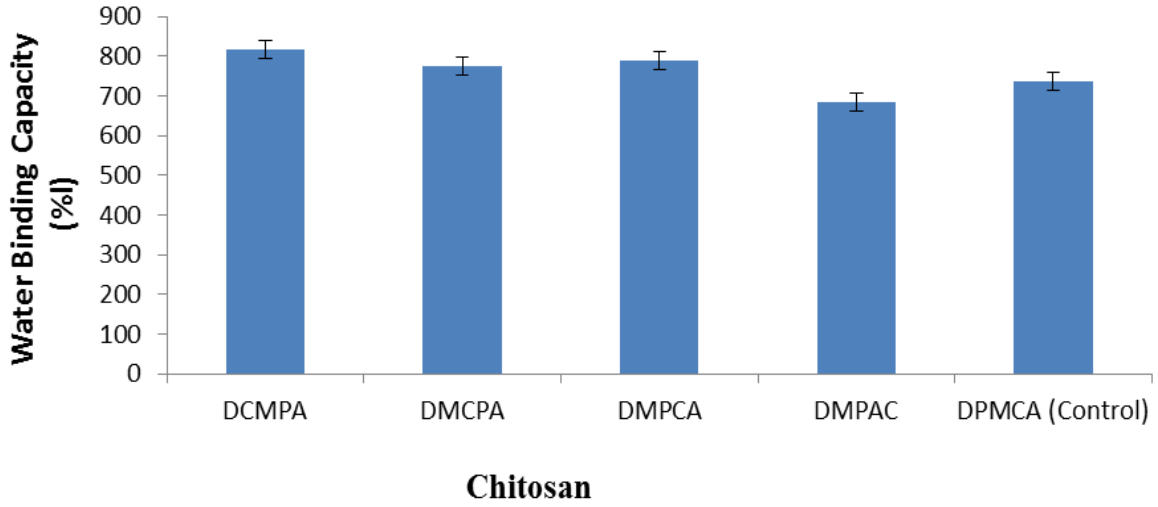

Figure 11. Binding capacity of water for chitosan from shells of snail with various modified process of production.
Source: Author

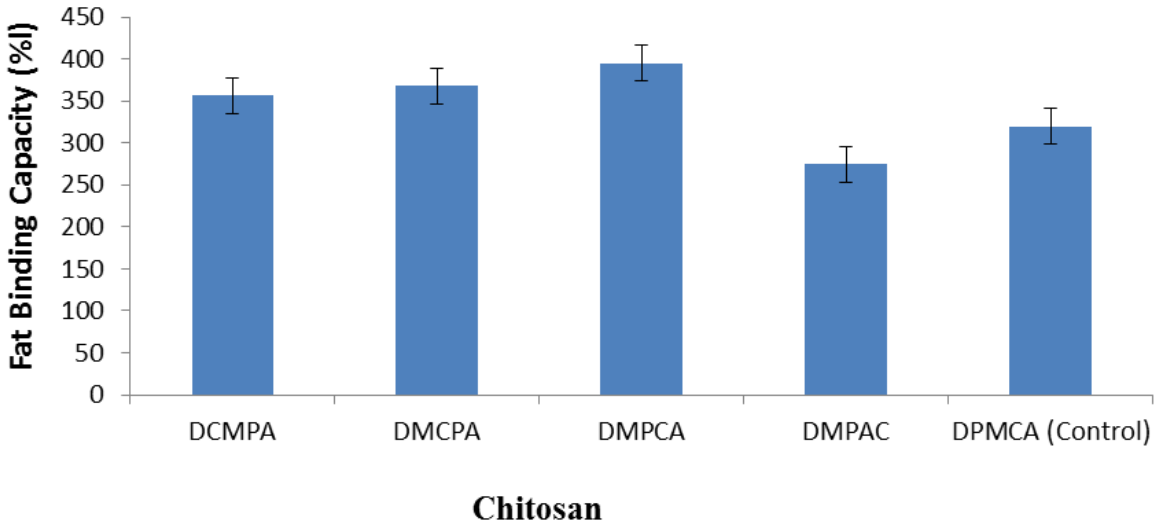

Figure 12. Capacity of fat binding for Chitosan produced from shells of snail with different process of sequence modification.
Source: Author

(Adekanmi et al., 2020b).

Conclusion

The current study made an effort to track the alteration of chitosan production procedures employing snail shell waste in order to ascertain whether such changes had any impact on the various physicochemical and functional qualities of chitosan. Using diverse methodological approaches, we altered or modified the process protocol, which had an impact on certain physicochemical and functional aspects of chitosan, as a result of the findings. Overall, the findings showed that process adjustment in the production of snail shell chitosan resulted in some differences in each feature over the control and commercial products. The interests of applications may differ from study to study and even from industry to industry, as was the case in the study, so it would be overstating it to say that just one modified technique is best for the production of chitosan. In light of the aforementioned, it is our recommendation that the

relationship between the process protocols/conditions and the resulting specific characteristics of chitosan products be properly and continuously monitored in order to achieve uniformity and proper product quality control for specific usage of chitosan. As a result, the current study will catch the interest of business people, industrialists, academicians, and environmentalists. Industries that process snails generate a lot of waste crustacean shell that is thrown out. By creating chitin and chitosan, this is a good waste management technique that raises the socioeconomic standing of coastal residents while also providing additional economic benefits. But because chitin and chitosan are biodegradable materials, they support environmental sustainability.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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